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The Effects of Dalapon on Pectic Substances and on Root Growth of Sugar Beets[1]

H. P. Alley, D. W. Bohmont and H. M. Hepworth[2]

Received for publication March 7,1960

Introduction

The herbicide, alpha, alpha, dichloropropionic acid, hereafter referred to as dalapon, is a selective chemical used for control of grasses. It has been used successfully as a post-emergent treatment for control of weeds in sugar beets. Although outstanding control has been obtained, the chemical has not been received with much favor. Considerable stunting of the sugar-beet seedling is prevalent, especially when treatments are made after the cotyledon to the 4-leaf stage of growth. Timely application is very important. Research data show no reduction in sugar-beet yield over a five-year period when timing was proper. This paper reports the results of a study conducted to: 1) determine the effect of post-emergent application of two rates of dalapon on the root growth of sugar beets; 2) determine by chemical analysis the effects of the dalapon upon the amounts of pectin and of pectic substances contained in sugar beets; 3) determine the longevity of inhibiting effects of chemical treatment upon sugar-beet roots.

Greenhouse Studies

As dalapon was known temporarily to delay sugar beet growth, a greenhouse study was undertaken to determine the reaction of the roots to post-emergent applications of the chemical at rates of 0, 6, and 10 pounds of acid per acre (hereafter referred to as pounds per acre). Galvanized metal flats were used which had been constructed so that a plate of glass served as one side to allow for observation of the root developments.

The flats were filled with sterilized, homogeneous, greenhouse soil and watered to field capacity and planted with sugar beet seed approximately ¾ inch deep. Shortly after emergence, the sugar beets were thinned to 6 plants per flat. The roots were marked and measured when the beets had reached the 2- to 4-leaf stage of growth. Dalapon was applied to 4 flats of plants each at 0, 6, and 10 pounds per acre.

Observations were made frequently and photographs were made weekly. Beet roots were measured in centimeters.

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[1] Published with approval of the Director, Wyoming Agricultural Experiment Station, as Journal Paper No. 146.

[2] Assistant Professor of Plant Science, Head of Division of Plant Science and graduate research assistant, respectively, at the College of Agriculture, University of Wyoming. Acknowledgement is made to Dr. R. J. McColloch, Agricultural Biochemistry Division, for the chemical analysis of the sugar beet samples.

Seventeen days after the chemical treatment, all of the plants receiving the 10-pound-per-acre rate were dead. At this point all plant roots were carefully washed from the soil for observation, photographing, and chemical analysis. One group of plants from the untreated and one from the 6-pound treatment were also washed for comparison.

Since all plants receiving 10 pounds of dalapon died, it was decided to repeat the experiment and treat the plants at an earlier stage of growth. In the next trial, the plants were treated shortly after the appearance of the first 2 true leaves 17 days after planting.

Fifty days after chemical application, the plants from one flat of the 6-pound treatment and one untreated flat were removed and the soil washed from them leaving the roots for comparison and chemical analysis of pectic substances. Since beets treated at the 10-pound rate had again died shortly after chemical application, no attempt was made to analyze these roots chemically.

Results of Greenhouse Study

Dalapon at 10 pounds per acre proved to be lethal to all treated sugar beets in the greenhouse trial. Within 3 days after treatment, all plants wilted. Foliage of beets treated with 6 pounds per acre displayed lack of vigor 3 days after treatment when compared to the check.

Root growth was retarded in the 6-pound-per-acre treatment compared to the untreated plants. Figure 1 illustrates the condition of the beets at time of treatment. Figure 2 compares the amount of root growth which had taken place during the week

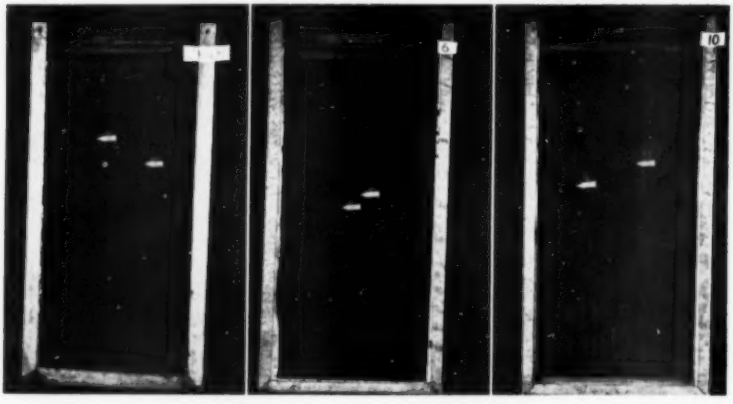

Figure 1.—Root development of sugar beets at time of treatment. Note root length compared with top growth, 17 days after planting.

Figure 2.—Root growth of sugar beets during first week after post-emergent application of dalapon at 0, 6, and 10 pounds per acre. Distance between arrows indicates the amount of elongation occurring 7 days after treatment.

after treatment. Roots of the untreated plants had grown 25 centimeters or more and had numerous lateral roots and root hairs. Dalapon at 6 pounds per acre had retarded root growth to 12 to 15 centimeters in a week with practically no lateral roots apparent. Root growth on beets treated at 10 pounds per acre was nil.

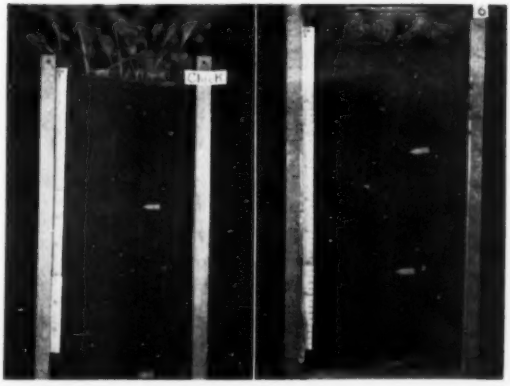

Figure 3.—Root growth 14 days after treatment with 6 pounds of dalapon per acre. Roots of treated plants have reached the bottom of the flats. Note the greater amount of lateral roots and root hairs on the untreated beets, left. Lack of top growth is apparent on the treated beets, right.

Fourteen days after treatment, roots of beets treated at 6 pounds per acre had reached maximum observable length. When compared with untreated plants, lack of development of both the roots and the tops was evident as seen in Figure 3.

The results of the chemical analysis for pectic substances are shown in Table 1. The percentage of pectic substances in alcohol-insoluble solids of untreated beets reached 13.52 compared with 9.82 and 2.73 for beets treated with 6 and 10 pounds of dalapon per acre. It appears that dalapon increases the percentage of alcohol-insoluble solids, but decreases the percentage of pectic substances in sugar beets for a time after treatment.

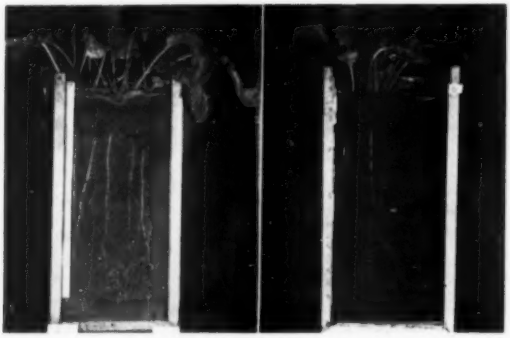

Figure 4.—Beets 40 days after treatment with dalapon at 6 pounds per acre. Lack of lateral roots and root hairs is still apparent in the treated beet, right. Foliar growth appears to be equal at this time.

In Figure 4 a flat of untreated plants is compared with a flat treated with 6 pounds per acre of dalapon 40 days after treatment. Apparently recovery had largely been completed by this time. Lateral roots and root hairs appear to be fewer on the treated plants. Washed roots from the flat in Figure 4 are compared in Figure 5. Results of chemical analysis for pectic substances in these beets, 50 days after treatment, are shown in Table 2. The amount of pectic substances in root tissues increased with maturity, as would be expected with sugar beets.

The amount of water-soluble pectic substances showed reversed trends in treated and untreated sugar beets. The percentage of water-soluble pectic substances in untreated beet foliage remained nearly equal during the 50-day period. The percentage of water-soluble pectic substances in the roots of untreated beets rose from 1.20 to 6.85 percent during the period. Dalapon-treated beets showed just the opposite condition with water-soluble pectic compounds rising from 1.00 to 5.59 percent in the

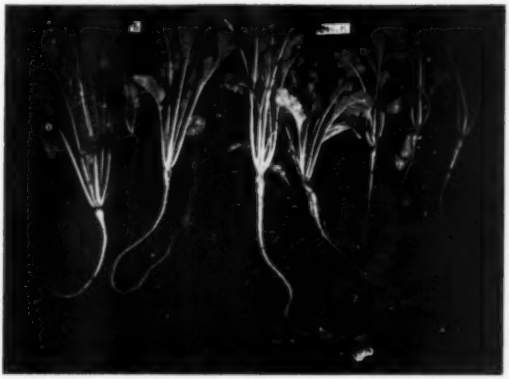

Figure 5.—Sample obtained for chemical analysis 50 days after treatment with 6 pounds of dalapon. Treated beets on the left appear to have recovered from effects of treatment at this date.

Table 1.—Chemical Analysis of Sugar Beets for Pectic Substances in Alcohol-Insoluble Solids.[1]

Sample	Rate of Dalapon Per Acre	Grams Fresh Weight	Percent AIS[2]	Percent Pectin in AIS				Percentage Pectin in Total AIS, Foliage, and Roots
				Water Soluble	Oxalate Soluble	Acid Soluble	Total	
Trial 1								
Foliage	0	46	3.36	0.06	0.96	5.15	6.17	5.71
Roots	0	9	5.10	0.15	2.81	1.24	4.20	
Foliage	6	5	4.70	0.00	6.43	0.00	6.43	4.80
Roots	6	1	8.00	0.00	0.38	0.00	0.38	
Foliage and roots[3]	10	10	10.60	0.00	2.45	0.28	2.73	2.73
Trial 2								
Foliage	0	190	2.46	2.70	9.30	2.90	14.90	13.52
Roots	0	20	5.20	1.20	3.80	2.20	7.30	
Foliage	6	41	3.47	1.00	7.40	2.90	11.30	9.82
Roots	6	3	32.80	2.40	2.60	2.70	7.70	

[1] Samples were analyzed 17 days after dalapon treatment.

[2] AIS refers to alcohol-insoluble solids; materials insoluble in 70 percent ethanol and water.

[3] Foliage and roots were combined because root sample alone was too small for analysis.

Table 2.—Chemical Analyses of Sugar Beets for Pectic Substances in Alcohol-Insoluble Solids.[1]

Sample	Rate of Dalapon Per Acre	Grams Fresh Weight	Percent AIS[2]	Percent Pectin in AIS				Percentage Pectin in Total AIS, Foliage and Roots
				Water Soluble	Oxalate Soluble	Acid Soluble	Total	
Foliage	0	73	9.99	2.47	3.61	10.84	16.92	16.39
Roots	0	56	5.40	6.85	2.66	5.77	15.28	
Foliage	6	82	9.92	5.59	2.14	9.10	16.83	15.89
Roots	6	25	5.74	2.97	2.12	6.13	11.22	

[1] Samples were analyzed 50 days after treatment with 6 pounds dalapon per acre.

[2] AIS refers to alcohol-insoluble solids; materials insoluble in 70 percent ethanol and water.

foliage, while the roots contained approximately the same amount on both dates (Tables 1 and 2). Indications are that dalapon interferes with the normal distribution of water-soluble pectic substances. The oxalate-soluble fraction of the pectic substances shows the same tendency to decrease in both treated and untreated sugar beets during the 50-day test period. The total amount of oxalate-soluble pectic substances is reduced by dalapon.

The percentage of acid-soluble pectic substances is smaller in beets treated with dalapon than in untreated beets. However, the decreased percentage of oxalate-soluble pectic substances is reversed and approximately equalled by the increase in percentage of acid-soluble pectic substances (Tables 1 and 2).

The percentage of alcohol-insoluble solids in root tissue is increased by dalapon treatments. This trend is consistent in all analyses (Tables 1 and 2). The effect of dalapon on the percentage of alcohol-insoluble solids in sugar beets is clearly noted. It is assumed that the percentage of alcohol-insolube solids rose from the time of treatment with dalapon to 32.8 percent at time of sampling. This assumption is based on the percentage of alcohol-insoluble solids in untreated plants the same age. Apparently as the effects of dalapon are overcome and root growth approaches normality, the percentage of alcohol-insoluble solids decreases to approximately that of untreated plants.

Field Study

Field studies were conducted at Wheatland and Torrington, Wyoming, in fields which had previously been treated with 6 and 10 pounds of dalapon per acre. The experimental plots were

selected at random from these larger fields. Twenty-one days, 28 days, 41 days, and 50 days after treatment, stand counts were made. Plants selected at random from each of the treated rows were given a figure rating from 1 to 10, with 10 indicating maximum plant size and vigor. The number of leaves on each of the 10 rated plants was counted and the length of the longest leaf on each rated plant recorded.

Excavation was made along the rows to expose the roots of the plants as they were growing in the soil. Length of roots was measured and photographs made.

Results of Field Study

Results of ratings and measurements made on plants in field plots at Wheatland 28 days after treatment are shown in Table 3. On a percentage basis the length of leaves on treated plants was reduced. The average height of beets in the plots treated at 10 pounds per acre was 80 percent of untreated plants. Six pounds of dalapon did not measurably reduce the vigor of the sugar beets, while 10 pounds reduced vigor by 41 percent. Analysis-of-variance treatment data show that leaf length is significantly reduced by 10 pounds of dalapon. Reduction in plant vigor resulting from treatment with 10 pounds of chemical per acre is significant at the 5 and the 1 percent levels.

Excavation of the root zone revealed little difference in the length of tap roots resulting from the various treatments after 28 days. Roots were measured to be 10.0 inches for untreated plants, 9.0 for the 6-pound dalapon treatment, and 8.5 for the 10-pounds-per-acre treatment. Closer examination revealed lack of lateral roots and root hairs as was observed in greenhouse trials. When removed from the soil for comparison, the roots on treated plants appeared definitely reduced in size and vigor, particularly the plants from the 10-pound treatments.

Table 3.—Effect of Two Rates of Dalapon Upon Sugar-Beet Vigor, Plant Heights, and Number of Leaves Compared with the Untreated Check at Wheatland.[1]

Treatment	Plant Height in Inches	Number of Leaves	Vigor[2]
Untreated	5.26	7.76	4.50
6 lbs. Dalapon	6.21	7.53	4.40
10 lbs. Dalapon	4.35	7.73	2.60
L.S.D. 5% Level	.70	NS	.87
L.S.D. 1% Level	NS	NS	1.16

[1] All figures are the means for 30 plants selected from each treatment.
[2] Vigor rating of 10 indicates maximum size and vigor.

Plots at Torrington 28 days after treatment were given vigor ratings of 10 for untreated beets, 7 for plots treated with 6 pounds per acre, and 4 plots receiving 10 pounds per acre. Stand counts were made on 25 feet of row replicated four times for each treatment. Average stand count for 25 feet of row was 20.8 plants for untreated rows, 23.8 beets in rows treated with 6 pounds of dalapon per acre, and 12.8 beets in the rows receiving 10 pounds of chemical. Analysis-of-variance treatment of the results shows that 10 pounds of dalapon reduced the stands significantly. An L.S.D. of 8.65 at the 5 percent level and 13.1 at the 1 percent level were required for significance.

Measurements of roots at Torrington 28 days after treatment failed to show any reduction in tap-root length of treated beets. However, the roots of beets treated with dalapon did not have the size or the profusion of lateral roots and root hairs exhibited by untreated beets. Only the beets treated at 10 pounds per acre showed a marked reduction in size when removed from the soil and compared with untreated beets. The effect of 6 pounds of dalapon appeared to be nearly outgrown at this stage. Comparisons of vigor, root weights, top weights, and root diameter at the crown are made in Figure 6.

Forty-one days after treatment, plots at Torrington were given vigor rating of 10 for untreated beets, 8 for 6 pounds dalapon, and 6 for 10-pound dalapon treatments. Forty-one

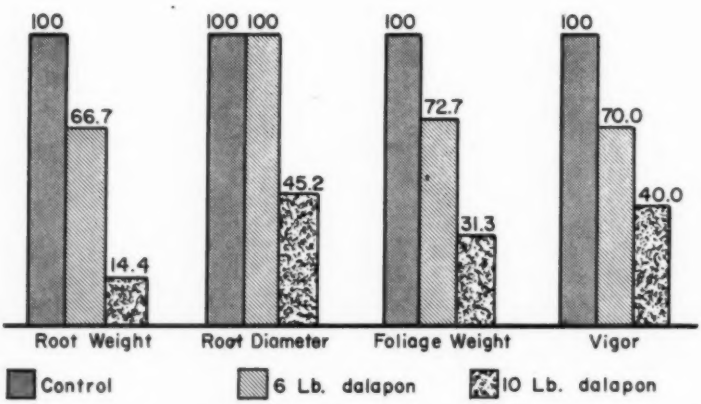

Figure 6.—Percentage comparison of root weight, root diameter at crown, foliage weight, and vigor of sugar beets from 3 treatments with dalapon. Observations made 41 days after chemical treatment. Untreated plants are considered as 100 percent.

days after treatment with 6 pounds of dalapon, beet roots would be about 60 percent as large as those not treated while beets given 10 pounds per acre are only one-third as well developed.

Samples taken at Wheatland 50 days after treatment showed the same pattern of growth. The foliage of the beets tends to return to normal before the root growth catches up. Size differences are apparent but length of roots appears equal. Pictures of 20 beets from each treatment are seen in Figure 7. As indicated by chemical analysis, it appears that by 50 days after treatment, the effects of dalapon applied at 6 pounds per acre have been overcome, but root growth of treated beets has not yet become equal to untreated beets.

Discussion and Summary

For at least 50 days after treatment with dalapon, sugar beets contain less pectic substances than untreated beets. The percentage of alcohol-insoluble solids in the roots of treated beets

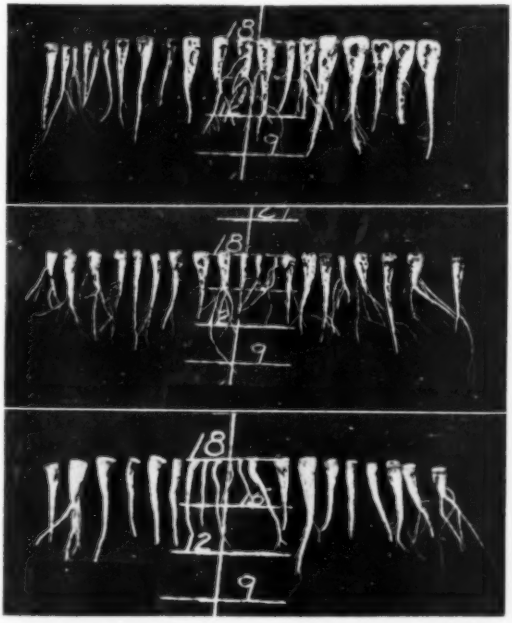

Figure 7.—Twenty beet roots from each of 3 dalapon treatments compared. Root length appears about equal in all samples. A reduction in size is apparent in the roots from the 10-pound treatment. Samples were taken 50 days after chemical application.

is much higher than in untreated beets. The cause for this is unknown, although one might postulate that, the pectic substance are believed to function in the transportation of water in the plants; therefore, if dalapon decreased the water-holding ability of the roots resulting in dehydration, then the percentage of alcohol-insoluble solids would be high.

Or, if the tops continue food manufacturing and translocation to root cells which are damaged by dalapon, there would again be an increase in the percentage of insoluble-alcohol solids. Perhaps cell walls are thicker, or the cell sap is more concentrated because of the upset in the balance of water-soluble pectic substances. As the effects of dalapon are outgrown by the plants and the root growth approaches normality, the percentages of alcohol-insoluble solids in the roots decrease to near that of untreated plants. Six pounds of dalapon per acre retarded the rate of root growth both in the greenhouse and in the field trials.

Field measurements indicate that 50 days after treatment with dalapon at 6 pounds per acre, foliar growth of beets was approximately equal to untreated beets. Root development was 60 percent as great in treated beets as in untreated beets. Since it has been found that the harvest yields are not reduced by 6 pounds of dalapon, it must be assumed that recovery is completed. Therefore, since good control of grassy weeds and some control of broadleaved weeds can be obtained with 6 pounds of dalapon, it would appear logical to use this rate as a post-emergent application.

Conclusions

1. Sugar-beet stands are significantly reduced with 10 pounds of dalapon per acre applied as a post-emergent spray.

2. Sugar beets that survive application of 10 pounds of dalapon are greatly retarded in root and foliar growth.

3. The percentage of alcohol-insoluble solids in sugar-beet roots increases rapidly for a time after treatment with dalapon. As the effects of dalapon are overcome by the growing plant, the percentage of alcohol-insoluble solids decreases to approximately that of untreated beets.

4. The amount of pectic substances in sugar beets is reduced for at least 50 days by treating with dalapon at 6 pounds per acre.

5. The amount of water-soluble pectic substances in alcohol-insoluble solids of sugar beets is affected by dalapon at 6 pounds per acre. The distribution of water-soluble pectic substances is

highest in the foliage of treated beets and in the roots of untreated beets. The oxalate-soluble fraction of pectic substances in alcohol-insoluble solids is smaller in dalapon-treated beets than in untreated beets. The percentage of oxalate-soluble pectic substances in alcohol-soluble solids decreases as the beets mature.

6. The acid-soluble fraction of pectic substances in alcohol-insoluble solids increases as the beets mature. Dalapon at 6 pounds per acre reduces the amount of acid-soluble pectic substances in treated beets compared with untreated beets

7. It may be expected that sugar beets treated with 6 pounds of dalapon as a post-emergent spray will be retarded in plant vigor, root, and foliar growth. However, one may expect treated plants to be equal in foliage to untreated plants after 50 days. Root growth of treated plants may be retarded somewhat longer.

Variety Climate Interactions of Sugar Beet Varieties in Simulated Climates[1]

ALBERT ULRICH[2]

Received for publication July 14, 1960

The question is often asked, will a sugar beet variety that is superior in one climate be superior in all climates, or just in one climate and not in another? Ordinarily, this question is answered by growing a series of sugar beet varieties in test plots in several locations during the same year and in different years. If some variety is found to be outstanding the first year, the tests are continued until the relative superiority of a variety is definitely established.

In conducting variety tests, the varieties are actually tested for an entire array of factors that include not only climate, but also disease, pest, soil and management factors as well. In a sense the answer to the question about the effect of climate on a variety is not a definitive one, but is confounded by factors not directly related to climate. Confounding of climate and disease factors can be clearly shown for resistant and non-resistant varieties in diseased areas, where all resistant varieties do better than non-resistant varieties and where their superiority is to a large extent independent of climate.

The real effects of climate on resistant and non-resistant varieties can be studied only by having disease-free conditions. An ideal place for such a study is in a phytotron (8)[3]. Here the environment can be kept free of diseases and insects, and in addition, the kind of climate can be controlled at will, an obvious advantage over waiting for nature to present the right climatic conditions necessary for making the proper experimental comparisons.

Procedure

The present experiments were conducted in the phytotron of the Earhart Plant Research Laboratory, California Institute of Technology, Pasadena 4, California (8). Four sugar beet varieties, US 22/3, US 35/2, European Kleinwanzleben E and ZZ, were compared in four simulated climates, cold, cool, warm and hot. The simulated climates were provided for in green-

[1] The present study was conducted at the Earhart Plant Research Laboratory, California Institute of Technology, Pasadena, California, by the University of California, College of Agriculture in cooperation with the California Institute of Technology and the Beet Sugar Development Foundation. The author is indebted to Professor Edgar Knapp, Einbeck, West Germany, for the sugar beet seed varieties Kleinwanzleben (Rabbethge and Giesecke) E and Kleinwanzleben (Rabbethge and Giesecke) ZZ.

[2] Plant Physiologist, Department of Soils and Plant Nutrition, University of California, Berkeley 4, California.

[3] Numbers in parentheses refer to literature cited.

house units that were illuminated by sunlight for natural day lengths of 9.9 to 13.1 hours. The greenhouse temperatures were kept at pre-set values from 8 AM to 4 PM and from 4 PM to 8 AM. These were for the "cold" climate, 17° C and 12° C; for the "cool" climate, 20° and 14° C; for the "warm" climate, 26° C and 20° C; and for the "hot" climate, 30° C and 22° C, respectively. The U.S. varieties 22/3 and 35/2 are resistant to curly top and the European varieties Klein E and Klein ZZ are non-resistant to curly top. Of these, the US 22/3 and the European Klein E are yield-type varieties and the US 35/2 and Klein ZZ varieties are sugar-type varieties, with the ZZ being an extreme sugar-type variety. The original selections for the US 22/3, US 35/2 and ZZ varieties were made to a large extent from the European Klein E, which in itself originated from many selections. Because of this common origin of genetic material it is of interest to compare the European Klein E with its "progeny" US 22/3, US 35/2 and ZZ as to performance in different simulated climates.

The seeds of the four sugar beet varieties were treated before planting with Phygon XL at the rate of 1% by weight. The pots were 4 gallons in capacity (25 cm in diameter and 30 cm deep), protected on the inside by a sanitary lacquer, and were provided with four small holes on the side at the bottom for drainage. The pots were filled with number 2 expanded vermiculite by adding successively small increments of the material to each pot in rotation. The vermiculite was moistened thoroughly with water and then settled firmly by jarring each pot several times on the floor. Four pots to a wheeled truck, 51 cm x 51 cm, were planted at random to the four sugar beet varieties, with one variety to a pot.

The seeds were planted on October 15, 1951, by placing 10 seedballs per pot in a circle 13.0 cm in diameter and forcing the seeds into the vermiculite to a depth of 2 cm. The seeds, and later the plants, were watered daily with Hoagland's culture solution (8); the excess solution drained off by gravity through the four drainage holes. During seed germination and early seedling development all trucks were kept in a greenhouse maintained at a temperature of 23° C from 8 AM to 4 PM and at 17 ° C from 4 PM to 8 AM. The seedlings, after 2 weeks, had developed to the early 2-leaf stage of growth and were then thinned to 1 seedling per seedball. On November 8, when the seedlings had progressed to the early 4-leaf stage they were thinned to 4 plants per pot. The following day the plants were moved to 4 simulated climates (Tables 1-6). In this move there

were 8 replications of each variety or a total of 8 trucks per climatic condition. The 8 trucks were randomized weekly within a climatic condition so as to minimize the variability due to greenhouse position. On November 23, 14 days after starting the differential climatic treatment, the plants were thinned to 2 plants per pot. The tops of the plants, which were removed by thinning, were dried at 80° C and then weighed. Old leaves, more than 50% necrotic, were removed and collected weekly beginning from January 7 until harvest on April 17, 1952.

The tops of the plants at harvest were separated from the roots just below the oldest living leaf. The crown material between the oldest living leaf and the first leaf scar was relatively small in amount and was included with the root for root weight and for sucrose analysis. The tops were separated into petioles and blades of "recently matured" leaves, blades of all remaining leaves and residue. Fresh weights were immediately recorded after the plants were harvested and dry weights were taken after the plant parts were dried in a forced draft oven at 80° C. All harvest weights were recorded on a pot basis. The beet roots were washed and wiped free of excess moisture, weighed and analyzed for sucrose by the hot water lead acetate extraction technique (1).

Differences between varieties and simulated climates were tested for significance by the method of analysis of variance. Pooled error variances were found to be uniform by Bartlett's test for only the sucrose percentages and leaf counts. For all other measurements the error variances were calculated from the replicates of the two treatments compared. Significance of the difference was estimated by the F-test, which in this instance is equivalent to the t-test, where $t = \sqrt{F}$ (5).

Results

Beet root weights. The beet root weights for the four varieties and four climates are given in Table 1. Here the beet root weights may be seen to be very uniform within the hot, warm and cool climates for the yield-type E and US 22/3 varieties, even though the weights doubled for the two varieties in going from the hot to the cool climates. However, in the cold climate the roots of the US 22/3 variety weighed only one-half of the European type E variety, indicating a variety-climate interaction in going from the cool to cold climates. Apparently, during the breeding program for curly-top resistance the ability to grow well in cold climates had been lost by the US 22/3 variety. This loss in ability to grow well in cold weather was not detected

in the field under curly-top conditions of the Salinas Valley, where spring weather is often as cold as in the simulated "cold climate."

The beet root weights of the sugar-type US 35/2 variety followed the same pattern as the yield-type US 22/3 variety (Table 1). It too produced only half the beet root weight of the E variety in the "cold climate." This again indicates a loss in ability to grow well in "cold climates" after breeding for curly-top resistance. As expected, the beet root weights for the sugar-type US 35/2 variety were in all instances less than for the yield-type US 22/3 variety but none of the differences within a climate were significant statistically. For the ZZ variety there was again a variety-climate interaction for a comparison of the ZZ to the E or of a U.S. variety in the cold and cool climates. The ratio of root weight for the E, US 22/3 or US 35/2 varieties to the ZZ variety within the cool climate is approximately 3 to 1. Whereas within the cold climate the ratio of E to ZZ is approximately 10 to 1 and for the U.S. to ZZ varieties, 5 to 1. The best climate for beet root growth is the "cool" climate (20° C days and 14° C nights) and the poorest is the "hot" climate (30° C days and 22° C nights.). On the average the E variety made the best beet root growth and the ZZ variety the poorest.

Table 1.—Effects of climate on *root weight* of yield- and sugar-type curly-top resistant non-resistant sugar beet varieties (Mean of 8 values expressed in counts per pot).

Simulated climate	Temperature		Yield-type		Sugar-type		Climate mean
	8 AM to 4 PM	4 PM to 8 AM	E	US 22/3	US 35/2	ZZ	
Cold	17° C	12° C	497 **	253 **	247 **	49	261
Cool	20° C	14° C	621	637	558 **	199	504
Warm	26° C	20° C	466 *	480 *	381 *	225	388
Hot	30° C	22° C	304	303	213	121	235
Variety mean	.....	.....	472	418	350	149	

The symbols * and ** indicate significant differences between the adjacent means at the 5% and 1% levels, respectively, calculated from the t-test for paired means (5).

Sucrose percentage. The sucrose percentages of the four sugar beet varieties followed the same general pattern that has been discovered in earlier work (6) namely, sucrose concentrations are higher in cold to cool climates and lower in warm to hot climates. The present values (Table 2) dropped from 11.71 to

Table 2.—Effects of climate on *sucrose percentage* of yield- and sugar-type curly-top resistant and non-resistant sugar beet varieties (Mean of 8 values).

Simulated climate	Temperature		Yield-type		Sugar-type			Climate mean
	8 AM to 4 PM	4 PM to 8 AM	E	US 22/3	US 35/2		ZZ	
Cold	17° C	12° C	11.5	10.8	11.0	**	13.7 **	11.71
Cool	20° C	14° C	10.3	10.0	10.9		11.9	10.76
Warm	26° C	20° C	9.2	9.1	9.8	**	11.6	9.94
Hot	30° C	22° C	8.4	9.4	9.4	**	12.0	9.81
Variety mean	.....	.....	9.81	9.83	10.27	**	12.31	.....

The least significant difference between climate or variety means equals 0.53% and 0.70% and between variety-climate means equals 1.1% and 1.4% at the 5% and 1% levels, respectively. The symbols * and ** indicate significant differences between the adjacent means at the 5% and 1% levels, respectively, calculated from the t-test for paired means (5).

9.81 from the cold to the hot climate. The yield- and sugar-type varieties performed as expected, i.e. the sugar-types tended to be higher in sucrose concentration than the corresponding yield-type. The differences, however, were relatively small and were not significant statistically for the U.S. varieties, but for the ZZ variety, which is an extreme sugar-type, the sucrose values were significantly higher than the E and U.S. varieties in all climates.

There was no significant variety climate interaction for sucrose concentration for the US 22/3, US 35/2 and E varieties, but for the ZZ variety, there was a significant variety-climate interaction for the cold and cool climate comparisons. The ZZ variety in the cold climate was relatively higher in sucrose concentration than in the cool climate, thus indicating that varieties may differ in relation to each other in different climates in sucrose concentration as well as in beet root size.

Sucrose weights. The sucrose weights for the four beet varieties in the four simulated climates are tabulated in Table 3. The variety-climate interaction is again significant for the yield-type varieties E and US 22/3, for sugar produced in the cold and cool climates. The sugar produced in the "cold" climate by the US 22/3 variety and also for the US 35/2 variety, is only one half of the E variety, whereas in the other climates there is no appreciable difference among the three varieties. The ZZ variety in comparison with the other varieties also seems to have produced proportionately less sugar in the "cold" climate than in the other climates. Maximum sugar production, as in beet root

weight, also occurred in the "cool" climate. The "fall off" in sugar produced by all varieties is quite appreciable in the "hot" climate and this loss should be studied in detail for the fundamental reasons why beet plants do poorly in hot weather.

Fresh weight of tops. The fresh weight of the tops (Table 4) offers no clue as to the reason for the poor growth of the roots of the U.S. curly-top resistant varieties in the cold climate. In fact, the tops of the U.S. varieties weigh as much as or even more than those of the E variety. This might imply that less

Table 3.—Effects of climate on *sucrose weight* of yield- and sugar-type curly-top resistant and non-resistant sugar beet varieties (Mean of 8 values expressed in grams per pot).

Simulated climate	Temperature: 8 AM to 4 PM	Temperature: 4 PM to 8 AM	Yield-type: E		Yield-type: US 22/3	Sugar-type: US 35/2		Sugar-type: ZZ	Climate mean
Cold	17° C	12° C	56.8	*	27.8	27.2	**	6.8	29.7
					*	**		**	
Cool	20° C	14° C	64.7		64.3	61.8	**	23.6	53.6
						*			
Warm	26° C	20° C	44.3		44.1	37.8		26.3	38.1
			*			*			
Hot	30° C	22° C	25.8		28.9	21.1		14.6	22.6
Variety mean	.....	.....	47.9		41.3	37.0		17.8	36.0

The symbols * and ** indicate significant differences between the adjacent means at the 5% and 1% levels, respectively, calculated from the t-test for paired means (5).

Table 4.—Effects of climate on *fresh weight of tops* of yield- and sugar-type curly-top resistant and non-resistant sugar beet varieties (Mean of 8 values expressed in grams per pot).

Simulated climate	Temperature: 8 AM to 4 PM	Temperature: 4 PM to 8 AM	Yield-type: E	Yield-type: US 22/3	Sugar-type: US 35/2		Sugar-type: ZZ	Climate mean
Cold	17° C	12° C	861	842	922	**	161	696
Cool	20° C	14° C	813	834	890	**	518	764
			*		*			
Warm	26° C	20° C	479	617	595	*	403	523
			*	*	**			
Hot	30° C	22° C	316	365	302		294	319
Variety mean	.....	.....	617	664	677		344	.....

The symbols * and ** indicate significant differences between the adjacent means at the 5% and 1% levels, respectively, calculated from the t-test for paired means (5).

sugar is produced by the leaves or translocated from the leaves to the roots by the resistant U.S. varieties than by the non-resistant E variety.

The tops of the ZZ variety, in sharp contrast to those of the US 22/3, US 35/2 and E varieties, weigh only one-third as much in the cold climate as in the cool climate (Table 4). This pronounced decrease in top weight for the ZZ variety results in a significant variety-climate interaction for top weight. The smaller tops for ZZ can account for the relatively smaller beet root weight for this variety (Table 1) but not for its higher sucrose concentration in the cold climate (Table 2).

The fresh weight of the tops dropped off appreciably in the warm climate and still more in the hot climate. These decreases in top weight are typical for sugar beet plants in warm climates (7). There were no significant variety-climate interactions in proceeding from the cool to the hot climate except possibly for the ZZ variety, which held up better in the hot climate than the other varieties (Table 4).

Dry weight of leaves. The amount and relative proportion of living, dead and combined living and dead leaves produced by the sugar beet plants differed considerably for the four varieties in the four simulated climates (Table 5). In the cold climate the dry weight of the living leaves made up about 75% of the total dry weight of living plus dead leaves produced, whereas in the cool climate the corresponding value for the living leaves was approximately 60%, in the warm climate 48% and in the hot climate 44%. In terms of living and dead leaves the living in the cold climate weighed approximately 3 times as much as the dead leaves, whereas in the hot climate the dead leaves weighed 30% more than the living leaves. The longer life expectancy of sugar beet leaves in the cold climate compensates for a slightly slower leaf initiation (Table 6), so that the living leaves in the cold climate weighed nearly as much as in the cool climate. Total leaf production (Table 5) and leaf count (Table 6) is greatest, however, in the cool climate.

An interaction of variety and climate is evident for the living leaf weight of the ZZ variety in the cold climate. The living leaf weight decreases sharply in the cold climate for this variety, whereas the weights increase appreciably for the other varieties. A similar interaction of variety and climate appears to exist for dead leaves formed by the ZZ variety in the cold climate. The dead leaf weight falls off much faster for the ZZ variety than for the other varieties.

Leaf counts. In terms of leaf counts (Table 6), the values are quite similar to those of total leaf weight. The total number of leaves produced to harvest did not differ greatly among climates. The lowest average value was 112 for the cold climate and the highest, 148, in the cool and warm climates. Again there were larger differences in numbers of living and dead leaves in relation to climate. In the cold climate the number of living leaves made up 61% of the total number of leaves produced

Table 5.—Effects of climate on *leaf weight* of yield- and sugar-type curly-top resistant and non-resistant sugar beet varieties (Mean of 8 values expressed in grams per pot on dry basis).

Simulated climate	Temperature		Yield-type		Sugar-type			Climate mean
	8 AM to 4 PM	4 PM to 8 AM	E	US 22/3	US 35/2		ZZ	
				Living Leaves				
Cold	17° C	12° C	91	85	97	**	21	73.6
							**	
Cool	20° C	14° C	76	78	88	*	58	77.9
Warm	26° C	20° C	47	59	61		46	53.2
				*	*			
Hot	30° C	22° C	33	35	30		35	33.5
Variety mean	.....	.....	63	64	69		40	58.8
				Dead Leaves				
Cold	17° C	12° C	32	30	32	**	12	25.2
			*	**	**		**	
Cool	20° C	14° C	54	53	62	**	33	50.4
							*	
Warm	26° C	20° C	55	62	61		47	56.3
					*			
Hot	30° C	22° C	47	50	43		35	43.7
Variety mean	.....	.....	47	49	32		50	44.2
				Total Leaves				
Cold	17° C	12° C	123	115	129	**	34	100.0
							**	
Cool	20° C	14° C	130	131	150	**	91	125.0
Warm	26° C	20° C	102	121	122		93	110.0
					**			
Hot	30° C	22° C	80	85	73		70	77.0
Variety mean	.....	.....	109	113	119		72	103.0

The symbols * and ** indicate significant differences between the adjacent means at the 5% and 1% levels, respectively, calculated from the t-test for paired means (5).

whereas in the hot climate the corresponding value was only 41%, a drop of nearly 50% in living leaf number relative to the total number of leaves produced.

Table 6.—Effects of climate on *leaf count* of yield- and sugar-type curly-top resistant and non-resistant sugar beet varieties (Mean of 8 values expressed in counts per pot.)

Simulated climate	Temperature		Yield-type			Sugar-type			Climate mean
	8 AM to 4 PM	4 PM to 8 AM	E		US 22/3	US 35/2		ZZ	
				Living Leaves					
Cold	17° C	12° C	68	*	80	69	*	55	68
								**	
Cool	20° C	14° C	70	*	84	75		76	76
					*	**			
Warm	26° C	20° C	65		70	** 54		65	63
			*		**				
Hot	30° C	22° C	51		51	49	*	64	54
Variety mean	.....	.....	64		71	62		65	65
				Dead Leaves					
Cold	17° C	12° C	43		51	50	**	32	44
			**		**	**		**	
Cool	20° C	14° C	77		76	80	**	59	71
					**			*	
Warm	26° C	20° C	83		93	89	*	74	84
Hot	30° C	22° C	78		89	80	**	64	78
Variety mean	.....	.....	69		77	75		57	70
				Total Leaves					
Cold	17° C	12° C	111	*	131	119	**	87	112
			**		**	**		**	
Cool	20° C	14° C	141	*	160	155	*	135	148
Warm	26° C	20° C	148		162	* 143		139	148
			*		*				
Hot	30° C	22° C	129		140	129		128	131
Variety mean	.....	.....	132		149	136		122	135

The error variances within groups were found to be homogeneous for living, old and total leaf counts by Bartlett's test (3). The corresponding error variances are 146, 143 and 364 and the least significant ranges for p = 2 at the 5% and 1% levels of significance for variety—climate means with eight replications and 112 degrees of freedom for error are 12, 12 and 19 and 16, 16 and 25, respectively. For the variety or climate means the least significant ranges for p = 2 at the 5% and 1% levels of significance are 6, 6 and 9 and 8, 8 and 13, respectively. The symbols * and ** indicate significant differences between adjacent means at the 5% and 1% levels, respectively.

Discussion

The results of the present experiment with four sugar beet varieties, resistant and non-resistant yield- and sugar-types, grown in four simulated climates, "cold," "cool," "warm" and "hot," suggest that the practice of comparing varieties at more than one location for more than one year is a good one if a sound evaluation of variety performance for sugar content and root size is to be made effectively. Varieties may parallel each other in performance for a fairly wide range of climates and then suddenly one or more of the varieties may fail to grow satisfactorily when the temperatures become slightly lower on the low side or slightly higher on the high side of the temperature scale. Under these conditions segregation of varieties, otherwise uniform, becomes immediately apparent. This phenomenon has been known of course for many genetic characters, but only recently appreciated for climatic factors as it influences genetic expression.

Recent germination studies at low temperatures also attest to the importance of getting sugar beet plants off to a good start during cold spring weather (4, 9, 10). That subsequent growth, particularly root growth, may also be retarded greatly in cold weather is not as well known. This reduction in root growth by one half is rather surprising since top growth, as measured by fresh weight, was not reduced at all. Apparently, the sugar produced by the leaves was either just sufficient to maintain top growth but not sufficient for maximum root growth, or perhaps the sugar synthesized in the tops failed to move out of the leaves into the roots for root growth.

What brought about the decline in ability of curly-top resistant varieties to form large roots during "cold" weather? One possible answer, although an unlikely one here, is that resistance and lack of ability to grow in "cold" weather are linked genetically. The present results provide no specific answer to this question. However, if we assume there is no genetic linkage or at least very little linkage between resistance and ability to grow in cold weather, one might postulate how this ability to form large roots in cold weather was lost. A review of the research program adopted for developing the curly-top resistant variety (2) indicates that the sugar beet seeds were planted, quite appropriately, at a time when the plants would be small and infestation by leaf hoppers would be high. Such a time is early summer, during warm weather. Under these conditions, however, it is not possible to select simultaneously for ability to grow in cold weather and disease resistance but only for disease re-

sistance. The ability to grow in cold weather just happened to be lost in warm weather, unless we are willing to assume that resistant varieties do not grow well in cold weather. This is a point in need of further study.

The failure to detect a loss of growth by resistant varieties in relatively cold weather from field experiments could well have been associated with two situations, one being that curly-top disease depressed the growth of the non-resistant varieties so much that their better growth in cold weather could not be detected and the other being that the duration of the cold weather was not always sufficiently long to cause a detectable loss of yield. A loss in yield of only 5% or even of 10% would be difficult to detect experimentally in the field even under disease-free conditions, but of course with curly-top disease present, the loss in cold weather could be masked completely. In the Salinas Valley a loss of only 5% would be approximately equivalent to 20,000 tons of beets per annum, which when translated to dollars would make it desirable to test "disease" resistant varieties for growth potential under "disease-free" conditions before the variety is released commercially.

Summary

Two yield-type sugar beet varieties curly-top resistant US 22/3 and non-resistant Kleinwanzleben E and two sugar-types, resistant US 35/2 and non-resistant Kleinwanzleben ZZ, were compared in four simulated climates by the open pot culture technique, using vermiculite flushed daily with complete Hoagland's nutrient solution. The greenhouse temperatures from 8 AM to 4 PM and 4 PM to 8 AM were kept for the "cold" climate at 17° C and 12° C; for the "cool" climate, 20° C and 14° C; for the "warm" climate, 26° C and 20° C; and for the "hot" climate 30° C and 22° C, respectively.

In the "cold" climate the US 22/3 and US 35/2 varieties produced only one-half as much storage root and sugar as Klein E, whereas in the "cool," "warm" and "hot" climates, there were no significant differences among the three varieties. Consequently, in the selection for curly-top resistance, ability for storage root growth in a "cold" climate had been lost by the US 22/3 and US 35/2 varieties. This variety-climate interaction for root growth and sugar produced by the three varieties did not carry over to top growth or to sucrose concentration. However, for the ZZ variety there was a variety-climate interaction for sucrose concentration and top size as well as for root size. Variety-climate interactions were also observed for leaf growth and leaf counts.

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A New Look at Some Nitrogen Relationships Affecting the Quality of Sugar Beets

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Nitrogen is an essential element for the growth of all plant life, including sugar beets. There has been a phenomenal increase in the rate of application of nitrogen fertilizers on sugar beets during the last two decades. Associated with this great increase in the use of nitrogen fertilizers and increased yields has been a decline in quality, and processing losses have increased. The term "quality" as used in this paper refers to the relative concentrations of impurities, with respect to sucrose, which interfere with the recovery of sugar. The influence of nitrogen fertilization on the decline in quality may be due more to the improper timing of nitrate uptake by the beet than by the total amount used in many cases.

The general trend of quality decline and its effect on processing losses were summarized at a general session of the American Society of Sugar Beet Technologists in 1958 and several reviews have been published since then (13, 22)[2]. Haddock, et al. (13) showed that the downward trend in sugar percentage of the sugar beet since 1937 was correlated with a much greater loss of sugar extracted from the beets. The average loss in extraction appears to have been more than five times as great as the decrease in sugar percentage. Although some increased losses in extraction are probably due to increases in the daily tonnage put through a given processing plant, the reduction in quality is undoubtedly the principal factor responsible for the decline in extraction.

The general trend toward higher yields with lower sugar percentage and lower quality suggests that yield and sugar percentage may be negatively correlated. On a given farm under uniform field practices other than nitrogen fertilization, this negative correlation is usually true; however, results between many farms within a district may not show this relationship. Frequently some farms having the highest yields produced sugar beets far above the average in sugar percentage. High yield, high sucrose percentage, and quality are evidently not incompatible but the factors responsible for their concomitant occurrence have not been clearly recognized.

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[2] Numbers in parentheses refer to literature cited.

A Major Cause of Low Quality

The depressing effect of high nitrogen nutrition on sugar beet quality was reported by Headden (14) as early as 1912. His report left little reason for doubt of the cause-and-effect relationship between high nitrate uptake and low quality. Later studies by Gardner and Robertson (9) also showed that excessive nitrogen reduced the sugar percentage and purity of sugar beets. Gardner and Robertson estimated that the reduction in sugar percentage was an approximately linear function of the nitrate nitrogen in the beet at harvest and that each 0.025 percent nitrate nitrogen in the beets reduced the sugar percentage by approximately 1 percent, which amounts to a 40 to 1 ratio between nitrate nitrogen and sugar percentage.

High nitrogen nutrition of sugar beets stimulates the growth of new leaves. Since leaves are the photosynthetic machinery that biochemically produces sugar from water and carbon dioxide for storage in the root, beets should have an abundant supply of nitrate to get them off to a good start early in their growth cycle (1, 25, 30, 33). Early development of a full canopy of foliage lengthens the time for the effective use of leaves for photosynthesis, thereby increasing sugar yields.

Nitrate uptake and metabolism require energy derived at the expense of sugar accumulation. First, energy is required to take nitrate into the roots (4, 5, 16, 20, 31) where its concentration is usually many times greater than that in the soil solution (4, 5). The energy for this accumulation of ions against a concentration gradient is derived from the respiratory metabolism of stored food, principally sugar. Second, nitrate must be reduced before it can be incorporated into usable compounds and this reduction requires eight hydrogen ions for each nitrate ion. Again the energy is derived at the expense of sugar accumulation, whether it is from the direct metabolism of sugar or from the photolysis of water (4, 5). Third, the ammonia formed by the reduction of nitrate, and possibly other partly reduced nitrate intermediates, is toxic to the plant (8, 23) and must be immediately combined with more than five times its weight (2) (% N $\times$ 6.25 = % protein) of photosynthesized sugar-forming intermediates to form amino acids which in turn are used for the synthesis of proteins and enzymes (4, 5, 24). Although these nitrogen-containing compounds are necessary for photosynthesis and other biochemical processes, they are impurities as far as the extraction of sugar is concerned (22) and sometimes they are produced in considerable excess of the amounts needed for optimum sugar production and storage.

Fife and Carsner (8) reported that "tip burn" occurs when sugar beets that have been grown in fertile soil with an abundance of nitrogen for a comparatively long time are subsequently grown under a low light intensity, and that the toxic substances involved are probably normal nitrogenous constituents of the plant temporarily in excessive concentrations. If the light intensity is too low to supply sufficient energy for rapid nitrate uptake, its reduction, and its subsequent combination with carbohydrates to form non-toxic amino acids, the reduced nitrogen compounds are toxic. Stout and Tolman (23) found that low concentrations of ammonia, if the concentration was maintained by constant flushing, were toxic to germinating sugar beet seeds and two-month-old plants.

High nitrate uptake causes a high rate of uptake of positively charged ions such as sodium and potassium in order to maintain electrical balance of cations and anions in the roots (16, 31). The data in Table 1 show that sugar beets heavily fertilized with nitrogen late in the season were much lower in sugar percentage and purity. They were also much higher in sodium, potassium, and amino nitrogen than normally fertilized sugar beets.

Table 1.—Effect of late, heavy application of nitrogen[1] on sugar beets, Gallinat plot, Salt Lake City, Utah, 1954.

Treatment	Average root wt.	Percent sucrose	Purity	Amino N	Sodium	Potassium
	Pounds		Percent	Percent	ppm	ppm
Heavy nitrogen	3.15	14.98	84.6	0.56	427	3318
Check	3.21	17.72	89.8	0.23	197	2369

[1] Nitrogen applied August 27, 1954, 300 pounds per acre of ammonium sulphate and September 10, 1954, 300 pounds of ammonium nitrate.

Variety SL 342.400. Above figures are average values of 20 individual beets of each nitrogen treatment.

Rounds, et al. (22) studied the effect of nitrogen nutrition and variety on processing losses. They found that high nitrogen nutrition caused increased impurities that reduced quality and extraction. Nitrogen compounds were highly correlated with non-sugars and ash, although different varieties responded somewhat differently to fertility levels.

Ulrich (28, 29) found that the sugar percentage of sugar beets when grown in full nutrient culture could be increased from about 7 to 12 percent by the best combination of day and night temperatures available at the Earhart Laboratory in Pasa-

dena, California. Sugar values above 18 percent were observed when nitrate was eliminated from the otherwise full nutrient medium for a time before harvest.

The roots of sugar beet plants are slow to enlarge until a fairly large canopy of leaves develops so that the leaves can capture enough sunlight and carbon dioxide to make more sugar than is required for new leaf growth and other metabolic processes. Only after this stage of development are the beets able to store an appreciable amount of sugar in their roots. Since an abundance of nitrogen stimulates the growth of new leaves, the plants apparently need a large amount of nitrogen early in the season to produce a rapid growth of foliar photosynthetic "equipment." For a period before harvest the nitrate supply should be greatly reduced to prevent the reinvestment of stored sugar in the production of surplus foliage. This regime of high nitrate uptake early in the season followed by a low rate of uptake before harvest should result in the maximum proportion of energy being used for photosynthesis and sugar storage and a minimum lost in excessive respiration, nitrate uptake, reduction and metabolism, and the production of excessive leaf growth.

The formation of an overabundance of leaves is not conducive to sugar accumulation in the root. Leaves are very porous to gases (5) and have a very high rate of respiration and transpiration in comparison with storage tissues. When leaves are exposed to light they normaly make more sugar than they consume. In the dark, especially at high temperatures, they burn up much of the sugar that is photosynthesized during the day. A surplus of leaves producing severe shading for some of them can cause the too densely shaded leaves to be a liability, rather than an asset.

In summarizing the best sequence of nutrients and environment for sugar beets, Went (33) stated that it should involve early nitrogen feeding at warm summer temperatures for maximum growth followed by low nitrate nutrition in sunny autum weather with night temperatures near freezing. The thermal requirements are fairly adequate in many of the sugar-beet-growing areas. Neither temperature nor light factors can be easily modified economically, therefore, the nitrate-nutritional regime is probably the most important factor subject to some measure of control.

The Possibility of Some Measure of Control of Nitrogen Uptake

Withholding nitrate from a culture solution is easy, but taking it out of the soil at the right time is more difficult. The method

generally used is to estimate the optimum amount to apply earlier in the season so that the nitrate will be depleted to the proper extent in the root zone at the right time. One problem frequently encountered in estimating nitrate requirements for sugar beets is the effect of incorporation of organic residues into the soil before planting the sugar beet crop. Tolman (25, 26) pointed out that the greatly increased number of micro-organisms that develop to decompose the organic material compete with the beets for available nitrate when the beets need it most. Late in the summer the micro-organisms begin to die and release nitrate when the supply to the beets should be greatly reduced.

Leaching nitrate from the root zone by heavy irrigation late in the summer would be wasteful of water and nitrate and, to be effective, would probably reduce aeration of the roots to the point where growth would be reduced and root rots would develop. Soil-profile nitrate studies (19) and preliminary studies by the writer of the nitrate content of irrigation and drainage waters have indicated that under normal irrigation practices leaching of nitrate from soils has been overestimated. Another, and not such an obvious approach to the reduction of nitrate uptake late in the season, is to help nature put the nitrate where the plants cannot get it—and save it for succeeding crops. This method has been in use since the beginning of agriculture especially in irrigated, arid climates such as those of western United States. Scientists have studied and given much concern to the movement of soluble salts in our soils (3, 18, 32) because these salts sometimes build up to such high concentrations as to threaten crop production in certain areas. The relationship between precipitation and evaporation is largely responsible for our saline soils and streams, but these same factors also affect the availability of plant nutrients. Nitrate is probably the most soluble anion in our soils and it is also the principal source of nitrogen taken up by plant roots.

In arid climates where there is little prospect of late-summer and fall rains, practices conducive to the redistribution of nitrate in the soil profile may be used to make some of the excess nitrate unavailable to the plants. In such areas the two nitrate-depleting factors, uptake by the roots and redistribution into dry surface soil, would complement each other. The extent of the evaporative redistribution of nitrate in the soil profile as a means of control of nitrate nutrition has not been fully recognized or utilized.

The tremendous amount of moisture that is evaporated from the surface of the soil is often ignored. Evaporation accounts for a large proportion of the total moisture applied to the soil by

rain or irrigation. Studies (17) at Rothamsted, England, with a 60-inch drain gauge over a number of years showed that over 53 percent of the average annual rainfall of 28.73 inches was evaporated from the soil surface (1871-1912). Hilgard (15) reported variations in annual evaporation values from a free water surface at ground level from 17.8 inches at Rothamsted, England, up to 108 inches at Calexico, California. The evaporation in the Salt Lake Valley, July, 1959, amounted to 14.2 inches. The same station recorded a total evaporation of 69.09 inches from April 1 to October 31, 1959.

No information on the moisture loss from a field of sugar beets is available, but Campbell, et al. (7) reported evapotranspiration values from sugar cane in Hawaii. They reported values of 0.37 tot 0.86 cm per day. The ratio of lysimiter evapotranspiration values to pan evaporation rate at cane-top height increased with the growth of the cane from initial values of about 0.4 to values averaging 1.1 for cane with well-developed canopies. The upward movement of moisture carries with it the soluble salts in the soil solution. The mobility of the salts or ions involved depends upon their concentration, solubility, and ability for ion exchange with other ions of relatively insoluble minerals in the soil. The high water-solubility of nitrate salts would probably preclude appreciable retention on the mineral constituents of the soil, but nitrates may be tied up rapidly by an increase in the number of microorganisms decomposing organic matter. In this case the nitrate is changed into organic-nitrogen compounds as well as rendered relatively immobile until the organisms die and the compounds are re-oxidized to nitrate.

Soil-profile nitrate and petiole-nitrate studies of growing plants can do much to evaluate the areas of nitrate concentration in the soil (18, 19) and the nutritional status of the plants (10, 11, 27). However, such studies can only evaluate, not modify the condition. As guides they can be used to develop modifications of cultural and irrigation methods to give some measure of control during the growing season. Late-summer or fall rains on beet fields frequently result in responses similar to those following an application of nitrate fertilizer. Haddock (12) found that sprinkler irrigation resulted in lower quality beets than applications of the same amount of water by furrow irrigation. Under sprinkler irrigation the petiole-nitrate values at harvest were more than four times as high as under furrow irrigation.

Many soils and petioles of beets have been tested for nitrate by means of diphenylamine reagent (27). Shortly before harvest the petioles frequently show little or no coloration with the reagent, but the dry soil in the ridges between beets is often

very high in nitrate. This sample qualitative test can give very useful and convincing information concerning nitrate nutrition and distribution. If the season has been dry the nitrate-redistribution pattern is evidently developed to the point where nitrate uptake by the plants is low. Obviously if the furrows are shallow a heavy rain or flood irrigation would upset this distribution pattern and result in high nitrate uptake, increased growth of leaves, and reduced sugar percentage and purity (6).

A quantitative laboratory study of nitrate redistribution was made by filling a 4-foot glass tube with well-mixed soil. Distilled water was supplied to the bottom of the tube and after the soil was moistened to the top, a 75-watt light bulb was placed 1 inch above the soil. Twenty days later the soil was removed in sections, dried, and analyzed. The total nitrogen concentration of the top inch of soil was ten times as high as that of the lower layers.

Soil samples were taken in a farmer's field of sugar beets near enough to a source of tap water so that a rain could be simulated by sprinkling if necessary. Even though the soil sampled in the ridge between furrows had been flooded at each irrigation, the data in Figure 1 (solid line) show a rapid decrease in total nitrogen content from the surface to lower layers. During the night following the first sampling and the next day, 0.46 inch of rainfall was recorded. Samples taken immediately after the rain, adjacent to and at the same elevation as the previous samples, showed a marked shift in the nitrogen content of the soil (broken line, Figure 1). Even less than 0.5 inch of rain had evidently shifted the maximum concentration from the surface to a depth of 4 or 5 inches.

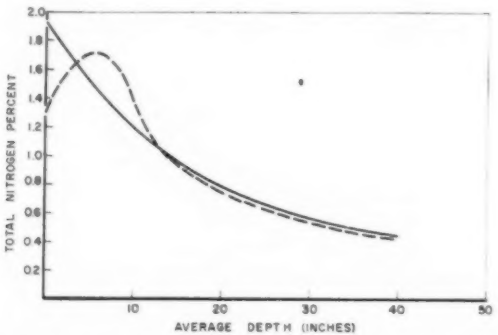

Figure 1.—Soil-profile nitrogen values before (solid line) and after a rain of 0.46 inches. Swensen Farm, Salt Lake Valley, October, 1957.

Data reported by Rost and Alway (21) showed that the surface 6-inch layers of three Minnesota soils contained 6 to 11 times as high concentration of nitrogen as the 25- to 36-inch layers.

McGeorge and Wharton (18) studied salt-concentration gradients in lettuce beds. The data showed that most soluble salts moved laterally toward the centers of the beds as a result of irrigation and upward with the evaporation of moisture from the surface. Nitrate moved more rapidly than other salts. Bernstein (3) also reported cross-sectional points of concentration of salts in various types of beds and explained why certain types are preferable for growing crops on saline soils under furrow irrigation.

The very high solubility of nitrates in comparison with that of other ions in the soil indicates that extreme redistribution of nitrate in the soil-profile might occur under arid conditions. Unpublished studies by the writer and also by R. A. Nielson of soils in sugar beet fields showed that nitrate concentrations in the top 0.5 inch of soil may be 500 or more times as high as at lower levels of the soil. This high concentration gradient was found in the dry surface soil between beets in the ridges of soil between furrows. Depletion of nitrate in the moist soil by the sugar beets as well as protection from rainfall by the foliage evidently contributed to the extremely high concentration gradients. Unprotected bare soil did not show this extreme concentration gradient after a rainfall. Immediately following a rain the nitrate concentration may be much higher in lower levels than in the surface of unprotected soils.

The extreme solubility and mobility of nitrate in soils with respect to moisture movement can be utilized in developing agronomic and irrigation methods to modify nitrate uptake by sugar beets in relation to the growing and harvest seasons. Many instances of high yields and high sugar percentage and quality have been reported. The influence of nitrate nutrition in relation to the seasonal needs of the sugar beet in order to produce maximum yields and quality is very important in sugar beet culture. Under different climatic conditions, other methods may be superior but the basic effect of these climatic factors on nitrate movement in the soil should be understood in order to develop better modifications of cultural practices. High ridges of soil in the rows of beets and foliar protection of the soil from rain may serve as an additional means of depleting the available nitrate late in the season in arid climates. With good foliar protection the nitrate in the surface may remain relatively unavailable fol-

lowing light rains. If, however, fall rains are heavy and frequent, the nitrate may become available to the plants at the least desirable period (6).

The principal purpose of the present report is to bring together some well-established basic facts concerning nitrogen metabolism and to point out that these facts have an important practical bearing on both the yield and quality of sugar beets. Proper timing of the relative rates of these basic processes is probably the key to a sound, economic solution. The effects of certain climatic conditions that modify the distribution of nitrate in the soil profile also affect nitrate uptake. This redistribution of salt concentrations in the soil profile has been studied extensively in relation to salinity problems. Concentration patterns shown by these studies have attractive possibilities for use in modifying the seasonal nutrition of the sugar beet. Figure 2 is a diagrammatic summary of some of these basic facts.

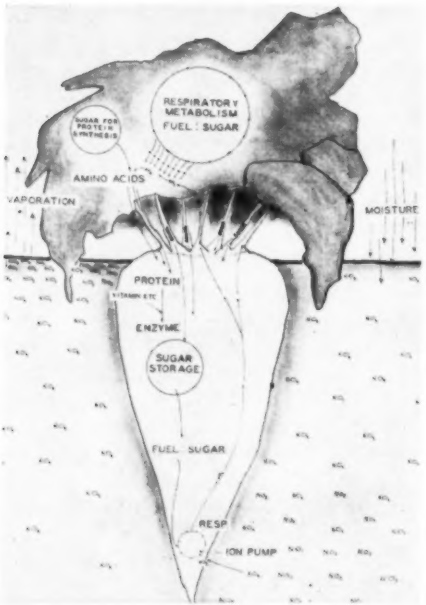

Figure 2.—Schematic diagram of nitrate uptake, reduction and metabolism in the sugar beet. Actual sites of processes figurative only. The effects of rainfall or irrigation and evaporation on nitrate distribution in the soil profile are indicated.

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An Examination of the Effectiveness of Various Sanitizing Agents against Yeasts Isolated from Liquid Sugar and Sucrose-Corn Syrup Blends

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In recent years, the market for liquid sugar and sucrose-corn syrup blends has greatly increased. Along with this market increase has come a relative increase in bacteriological and mycological problems associated with the preparation and distribution of these products. Yeasts have been isolated which grow readily at sugar-blend concentrations of 72 to 75 Brix. Once these yeasts contaminate mixing tanks, storage tanks and transmission lines, it is difficult to sanitize all areas which contact the sugar products to eliminate contamination which will again seed the next batch of product produced.

The purpose of the investigation to be reported by this paper has been twofold: First, to test the activity of various sanitizing agents against three different colonial types of yeast isolated from liquid sugar products, and second, to compare the sanitizing efficiency of these agents as tested under laboratory conditions.

Materials and Methods

Yeasts: Three distinctively different colonial types of yeast have been isolated from liquid sugars and liquid sugar blends during routine microbiological examination of submitted samples. The yeast most commonly found in blends grows as a small white colony on mycophil agar. Usually the number of microorganisms per 10 grams of dissolved sugar equivalent is high because this organism will multiply in the high concentrations of carbohydrate involved (Figure 1). A second yeast, seen less often, grows anaerobically in a "star burst" type of colony (Figure 2). At the surface, under aerobic conditions, the colonial form is fluid and thinly mucoid in its early stages of growth. The colony enlarges rapidly, and if the agar plate is tipped at an angle of more than 30° from the horizontal, the colony will flow across the agar surface. Upon standing, the colony loses it fluid characteristic and develops a black pigment. This pigment first appears as a ring at or near the center of the colony, and then as the culture ages the pigment extends throughout the entire colonial mass.

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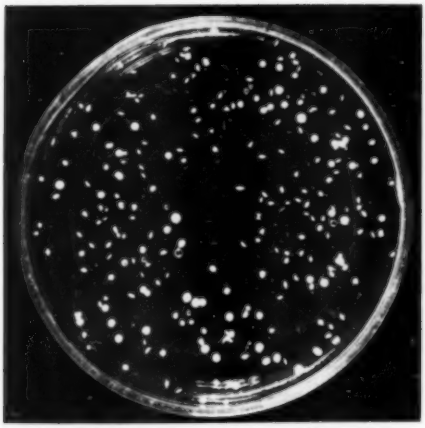

Figure 1.—Colonial morphology of first type yeast.

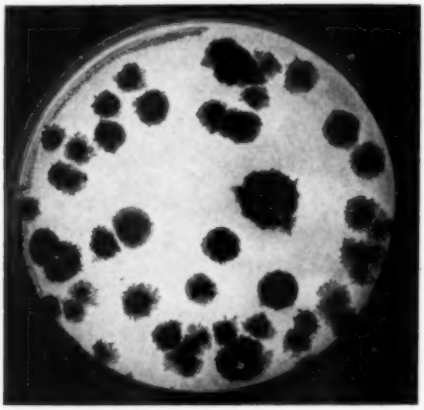

Figure 2.—Colonial morphology of second type yeast.

The frequency of the appearance of the black yeast is greatest in the summer months, and has been seen very infrequently during the winter months.

The third yeast has been observed with some regularity in routine samples, but seldom in large numbers. It appears to be confined to sucrose syrups. It is possible that this colonial type is the same as the first organism, but shows the different characteristics in the different environment.

The first described yeast was used almost exclusively for the germicidal efficiency tests. All three were tested for sensitivity to germicide.

A stock culture of each yeast type was maintained on acidified mycophil agar slants. All stock cultures were stored at 4° C. Test cultures were grown in mycophil broth for 24 hours at room temperature (22° C ± 5°) and at a pH of 4.8 to 5.2.

All tests were carried out with 24-hour yeast culture suspensions.

Germicidal Efficiency Test: The germicidal efficiency of the several sanitizing agents as well as the sensitivity of the yeast cultures to the agents was tested by the method of Chambers (1)[2].

Each agent was prepared at a predetermined concentration and 100 ml placed in a 250 ml Erlenmeyer flask. One ml of the 24-hour broth yeast suspension was added to the flask. Fifteen seconds later and at intervals of 30 seconds, 1, 2, 5, 10, 20 and 30 minutes, a one ml portion of the germicide-culture mixture was removed and placed into 9.0 ml of neutralizer which consisted of lecithin suspended in a solution of Tween 80. Thus the 1 ml aliquot samples were removed and transferred to neutralizer after 15 and 30 seconds, and 1, 2, 5, 10, 20, and 30 minutes exposure to the sanitizing agent.

As soon as possible after removal of the aliquots to the neutralizer, 1 ml of the contaminated neutralizer was transferred to each of two tubes of mycophil broth. The latter were incubated for a period of 72 hours at room temperature (22° C ± 5°) and then examined for yeast growth. The results of tests are reported as killing effect after an exposure of a given time.

The effect of the neutralizer on the test organism was observed by adding 1 ml of culture to 100 ml of sterile distilled water. After thorough mixing, 1 ml of diluted culture was transferred to a tube of neutralizer. One ml of neutralizer culture suspension was then transferred to each of two tubes of mycophil broth. Two more tubes of mycophil broth were inoculated from the same neutralizer culture suspension after completion of the test run. Growth was always positive in all tubes.

The effectiveness of the neutralizer was observed by adding 1 ml of sanitizing agent to 9.0 ml of neutralizer. This was then inoculated immediately with yeast culture and 1.0 ml aliquots transferred to mycophil broth. Growth was always positive in all control tubes and it appears that the lecithin in Tween 80 was equally effective as a neutralizer against the iodine compounds as well as the quaternary ammonium compounds.

It was shown that added sucrose decreased the killing effectiveness of both Hyamine and Weladyne (see results). The following titrimetric methods were carried out to show that this

[2] Numbers in parentheses refer to literature cited.

effect was accompanied by a loss in titratable iodine. The effect of sucrose on titratable Hyamine 3500 was also tested but the results were equivocal. The carbohydrate interfered with the argentimetric titration; therefore, no results are reported.

Zero, 10, 20, 30, 40 and 50 grams of sucrose were added respectively to 100 ml of a solution of 100 ppm (9.8×10^{-4} N) Weladyne in distilled water. Each of these solution mixtures was added from a burette into 5 ml of 0.001 N Sodium thiosulfate in which starch had been added as an indicator.

It was recognized that volume error was introduced into the above titrations. Therefore in a second experiment, a different volume of sugar in each case was added to a given volume of iodine solution. Another titration was carried out as above and the volume effect of added sugar taken into account. A second solution containing 100 ppm of available iodine was prepared and percentile solutions of sugar at 0, 10, 25, 35, and 50% were prepared with the iodine solution. Each of these was titrated into 5 cc of 0.001 N Sodium thiosulfate.

Results

Ten different germicidal agents were first tested at concentrations recommended by the manufacturers. In all experiments in the absence of added carbohydrate, all agents were effective and test organisms were killed in less than a 15-second exposure.

Many of these agents were then tested at concentrations of 5 and 10 ppm. The iodine compounds at this low concentration were much superior to the quaternary ammonium compounds.

After scanning several agents, two which demonstrated the greatest effectiveness in both classes of compounds were settled upon for more intensive study, namely Hyamine 3500[3], a quaternary ammonium compound, and Weladyne[4], an iodine-detergent complex. The comparative effectiveness of these two compounds is demonstrated in Table 1. The effectiveness of the iodine complex at 10 ppm. titratable iodine, is comparable to that of the quaternary ammonium compound at 200 ppm.

As it was proposed that these agents were to be used to sanitize tanks and transmission lines where rather large residual amounts of sucrose and sucrose syrups would be extant, it was imperative that the agents be germicidal in the presence of rather high concentrations of carbohydate. Hyamine 3500 was tested, first at 100 ppm in 5% and 10% sucrose solution and then at 200 ppm in 5%, 10% and 15% sucrose solutions with

[3] Hyamine 3500 supplied from stocks of the Amalgamated Sugar Company. The compound is manufactured by Rohm and Haas, Inc., Philadelphia, Penn.

[4] Weladyne supplied by the Chilean Iodine Information Bureau. The compound is manufactured by the West Chemical Co., Long Island City, N.Y.

Table 1.—A comparison of two sanitizing agents.

Germicide	Germicide concentration ppm	Exposure time to kill
Hyamine 3500	20	30 min
	40	20 min
	60	10 min
	80	10 min
	100	2 min
	200	15 sec
Weladyne	5	2 min
	10	15 sec
	50	15 sec
	80	15 sec

Table 2.—The effect of sucrose on the germicidal efficiency of Hyamine 3500 and Weladyne.

Germicide	Germicide concentration ppm	Sucrose concentration %	Exposure time to kill
Hyamine 3500	100	5	2 min
	100	10	5 min
	200	5	1 min
	200	10	2 min
	200	15	5 min
Weladyne	50	5	15 sec
	50	10	15 sec
	50	20	15 sec
	50	30	30 sec

the results shown in Table 2. The activity is markedly reduced as compared with solutions containing no carbohydrate. Also, as the carbohydrate is increased, the activity of the agent decreases.

Weladyne was also examined at 50 ppm in the presence of 5, 10, 20, and 30% sucrose solutions with results as presented in Table 2.

It is obvious that the iodine-detergent compound was much less affected at comparable concentrations of sucrose than was the quaternary ammonium compound.

These two compounds were further examined by maintaining the sugar concentration at 50% and the concentration of the agents varied from 40 through 100 ppm in increments of 10 ppm. The result is shown in Table 3. The iodine complex again was shown to be less affected by the very high concentration of carbohydrate.

Table 3.—A comparison of the germicidal properties of Hyamine 3500 and Weladyne in the presence of 50% sucrose.

Germicide	Germicide concentration ppm	Sucrose concentration %	Exposure time to kill
Hyamine 3500	50	50	30 min
	60	50	30 min
	70	50	30 min
	80	50	20 min
	100	50	20 min
Weladyne	50	50	30 min
	60	50	30 min
	70	50	20 min
	80	50	5 min
	100	50	2 min

Germicidal tests against the "black yeast" showed comparable results except the "black yeast" was more sensitive to the sanitizing agents than the yeast found regularly in blend. Tests for sensitivity of the third yeast to germicides indicate it to have a sensitivity comparable to that of the first yeast described.

The results of the two iodine titrations are graphically presented in Figures 3 and 4. By adding 50 grams of sugar to 100 ml of solution, the normality of the iodine dropped from 9.8×10^{-4} N to 5.0×10^{-4} N, a loss of half the available iodine. Where more sugar was added per unit volume of solution, as demonstrated by preparing percentile dilutions of sugar, the fall off in titratable iodine was much more precipitous (from 10.8×10^{-4} N to 1.8×10^{-4} N).

It was also shown by further laboratory experimentation that both Hyamine 3500 and Weladyne would deteriorate if stored in open vessels at dilutions recommended for use as sanitizing agents. The chemically titratable activity is lost and the germi-

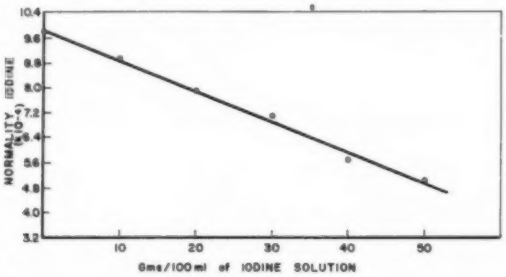

Figure 3.—The effect of sucrose on titratable iodine.

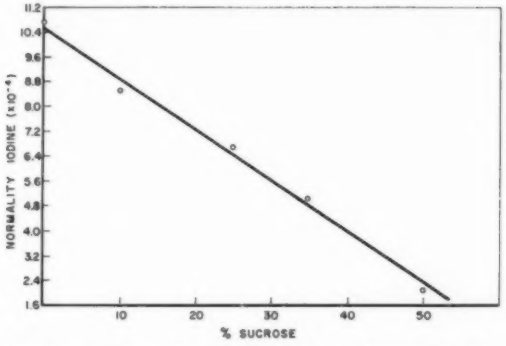

Figure 4.—The effect of sucrose on titratable iodine.

cidal efficiency of both is impaired. Further, the rapidity of deterioration is increased if the storage temperature is higher than 22° C.

Discussion and Conclusions

It has been demonstrated that yeasts commonly found as contaminants in liquid sugar syrups and in liquid sugar-corn syrup blends are readily killed in recommended concentrations of several different sanitizing agents at present on the market. It was also demonstrated that two of these, Hyamine 3500 and Weladyne were especially active, even in the presence of sucrose, but the efficiency of both was impaired. The carbohydrate affected the iodine compound (Weladyne) less than it did the quaternary ammonium compound (Hyamine 3500).

It was also shown that both the iodine and quaternary ammonium compound in water solutions deteriorated upon standing and that the rate of deterioration was affected by temperature. As would be expected, warm temperatures increased the rate of deterioration. Iodine compounds were less stable than the quaternary ammonium compounds, but have the advantage of a built-in indicator. As long as color is present—the iodine is relatively active. One can therefore "see" whether or not the sanitizing agent is still at active concentration.

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The Effect of Phosphorus Fertilization and Time of Application on Chemical Composition of Foliage and on Yield, Sucrose Content and Percent Purity of Sugar Beet Roots[1]

J. F. Davis, Grant Nichol and Don Thurlow[2]

Received for publication February 5, 1960

Michigan farmers use approximately 600 pounds of fertilizer per acre annually for their sugar beet crop. On the average, the most practical application is within the range of 100 and 150 pounds of P_2O_5 and 50 to 100 pounds of K_2O annually. In addition to causing increases in yield of roots, fertilizers have a marked stimulative effect on the early growth of the young plant. This latter effect is largely from the phosphate applied. Furthermore, there is some indication that phosphate fertilizers, particularly when banded close to the seed, will decrease the incidence of certain black root organisms. By stimulating the early growth of the seedlings, the beets can be blocked and thinned earlier resulting in a more economical use of labor. Earlier cultivation can be a factor in minimizing the weed control problem. Weather conditions, because of seasonal rains, may allow for only a few days of ideal planting weather. A rain can result in delayed planting and, in turn, reduced yields. If the amount of fertilizer applied at planting time could be reduced without a practical decrease in the rate of early growth and final yield, a substantial decrease in the spring labor requirement would result.

The objectives of the present study were to determine 1) the minimum amount of fertilizer to be applied at planting time in relation to the total application without significantly decreasing early growth or final yield of roots, and 2) the amount of preplant phosphate required to best complement the planting time fertilizer.

Materials and Methods

A rotation of beans, beets, and wheat, with a companion crop of sweet clover in the wheat was established near Bay City, Michigan, on a Kawkawlin loam soil. Each crop in the rotation appeared each year. For the sugar beet crop, 4 rates of P_2O_5 were broadcast ahead of planting: 0, 200, 400, and 800 pounds

[1] Contribution from the Soil Science Department, Michigan Agricultural Experiment Station, East Lansing, Michigan, and Agricultural Department, Monitor Sugar Company, Bay City, Michigan, and approved by the director as Journal Article No. 2581.

[2] Professor of Soil Science, Michigan State University, Agronomist, Monitor Sugar Company, and Assistant Instructor, Michigan State University, respectively.

per acre. A basic application of 200 pounds of 60 percent of muriate of potash was plowed under. Treatments were replicated 3 times in an east-west direction of the field. Superimposed on these areas were 3 rates of fertilizer applied 1 inch to the side and 2 inches below the seed at planting time at rates of 0, 150 and 300 pounds of 5-20-10 per acre. The 5-20-10 fertilizer contained 2 percent manganese and 1½ percent boron. The rows were planted across the plots where the P_2O_5 was broadcast. The planting time fertilizer applications were replicated 4 times. The beans and wheat received 150 pounds per acre of 5-20-10.

The plot size was four 28-inch rows by 66 feet. Monogerm beet seed, variety SL108 x SP5481, was planted May 7 and stand counts were taken May 22 and June 12. Plant samples of 100 plants per plot were taken June 12, oven dried at 65° C, weighed and analyzed for phosphorus, potassium, calcium, and magnesium. Soil samples were taken in the spring before the fertilizer was applied and again in August. The soil samples were analyzed for phosphorus using .025 N HCl + .003 N NH_4Fl extractant. Potassium was determined from a 0.13 N HCl extracting reagent.

Petiole samples were taken July 15 and September 1 and a portion of each sample was extracted with a 10% sodium acetate in 3% acetic acid solution (1:20 ratio of green tissue to solution), and the percent phosphorus determined. The remainder of the tissue was dried and analyzed for total calcium and phosphorus using a perchloric acid digestion procedure.

The beets were harvested November 10 and the number, yield, percent sucrose, and percent apparent purity were determined.

Results and Discussion

The effect of fertilizer treatment on the weight and chemical composition of the plant samples taken June 12 are recorded in Table 1. There was no effect of fertilizers noted on stand.

Fertilizer applied at planting time increased the early growth of the plant, the percent phosphosus in the tissue, and the uptake of phosphorus. Phosphate plowed down caused similar effects. The greatest effect in planting time fertilizer both in regard to weight and percent phosphorus in the plants was obtained at the lower amounts of the preplanting application of phosphate. The values per 100 plants ranged from 34.0 to 102.0 grams dry weight, .356 to .625 percent phosphorus, and .121 to .638 grams of phosphorus taken up.

The potassium applied at planting time increased the percent and total amount of potassium in the plant. The effect was more pronounced where the lower rates of phosphate had been

Table 1.—The effect of time and method of application of fertilizers on the early growth of sugar beets and uptake of nutrients. (Monitor Sugar Co., 1959).

Lbs./acre P_2O_5 plowed down —spring	Lbs./acre 5-20-10 planting time	Grams per 100 plants at blocking time	Percent P in tissue[1]	Grams P per 100 plants	Percent K in tissue[1]	Grams K per 100 plants	Percent Mg in tissue[1]	Grams Mg per 100 plants	Percent Ca in tissue[1]	Grams Ca per 100 plants
0	0	34.0	.356	.121	6.66	2.26	.71	.241	.803	.273
0	150	68.6	.430	.295	9 97	6.84	.82	.563	.904	.642
0	300	78.0	.458	.357	10.86	8.47	.89	.694	.928	.724
200	0	56.3	.479	.276	8.43	4.75	.76	.428	.863	.486
200	150	78.5	.521	.409	9.18	7.20	.87	.683	.970	.762
200	300	91.1	.527	.480	11.76	10.71	.82	.747	.968	.882
400	0	67.6	.513	.347	10.74	7.26	.82	.544	1.005	.679
400	150	84.9	.576	.489	11.24	9.54	.92	.790	.997	.845
400	300	95.0	.584	.555	10.78	10.24	1.01	.960	.939	.892
800	0	79.1	.588	.465	11.22	8.87	.82	.649	.863	.685
800	150	99.2	.616	.611	9.37	9.29	.78	.684	.902	.895
800	300	102.0	.625	.638	11.93	12.16	.84	.857	.898	.916
L.S.D. 5% Level:										
Rates P_2O_5		15.76	.076		1.73		NS		NS	
Rates 5-20-10		4.41	.029		1.57		.087		.055	

[1] Oven dry

applied. There were no significant differences in either percent magnesium or percent calcium in the tissue, although the amounts of magnesium and calcium taken up by the plants increased with the yield.

These data indicate that in order to get maximum early growth of the plant, some fertilizer at planting time should be applied. They also show that the early growth of the plant is increased if sufficient P_2O_5 is broadcast and plowed under ahead of planting. For example, the plants from the plots receiving 300 pounds of planting time fertilizer weighed 78 grams in contrast to 34.0 grams where no planting time fertilizer was used. The respective weights for 400 pounds of P_2O_5 plowed under and 800 pounds plowed under were 67.6 and 79.1 grams. Although the growth of the plants tended to increase in weight with the amount of planting time fertilizer applied, the results indicate that it is not necessary to apply more than 150 pounds of fertilizer at planting time, provided the phosphorus level in the soil is sufficiently high.

Data in Table 2 show that on the July 15 sampling date as the amounts of phosphate plowed under increased, the percent extractable phosphorus in the green petioles increased. The

Table 2.—The effect of time and method of application of fertilizers on the chemical composition of leaf petioles at two sampling dates.[1] (Monitor Sugar Co., 1959).

Lbs./acre P_2O_5 plowed down —spring	Lbs./acre 5-20-10 planting time	Beet sample taken July 15			Beet sample taken Sept. 1		
		P in green tissue	Total P	Total Ca	P in green tissue	Total P	Total Ca
		percent	percent	percent	percent	percent	percent
0	0	.124	.180	.97	.089	.110	1.15
0	150	.129	.167	.90	.079	.114	1.41
0	300	.168	.195	1.00	.120	.146	1.20
200	0	.173	.220	.98	.215	.236	.97
200	150	.195	.231	.88	.189	.208	1.10
200	300	.184	.224	1.02	.168	.218	1.17
400	0	.193	.237	.93	.248	.256	.93
400	150	.204	.249	.83	.229	.217	1.01
400	300	.191	.240	.96	.205	.243	.91
800	0	.216	.243	.95	.242	.234	.88
800	150	.219	.260	.80	.223	.232	.91
800	300	.223	.267	1.05	.230	.247	.93
L.S.D. 5% level:							
P levels		.030	.026	NS	.040	.026	.16
Planting time		NS	NS	.06	.017	NS	NS

[1] Green tissue extracted with a 10% sodium acetate in 3% acetic acid solution (1.20 ratio of green tissue to solution). All values reported on an oven-dry weight basis.

largest increase in extractable and total phosphorus from the planting time fertilizer occurred where the 0 and 200 pounds of phosphate per acre were plowed down.

Beets sampled September 1 showed similar trends as those sampled July 15. There was some indication, however, that the extractable phosphorus in the plants where planting time fertilizer only was used was lower on the September 1 sampling than on the July 15 sampling. The phosphorus contents of the petioles increased where phosphate had been plowed under ahead of planting. This indicates that the phosphate supply for the beet plants was insufficient where no phosphate had been plowed under. The comparison was not as clear-cut for the total phosphorus content as for extractable phosphorus in the green tissue. Approximately 85 percent of the total phosphorus in the plant was accounted for by the extractant used. The percent of total calcium found in the tissue July 15 was lower than that found on September 1. There was a tendency for the calcium content of tissue to decrease where 800 pounds per acre of P_2O_5 was plowed down.

These data show that at low residual fertility levels, the amount of phosphate applied at planting time significantly increased the extractable phosphorus content in the green tissue of the plant. However, if there was sufficient phosphate fertilizer in the soil the effect of planting time fertilizer on the phosphorus content of tissue was largely masked.

The sugar beet crop responded very well to both planting time fertilizer and phosphate plowed down (Table 3). It was only at the zero level of phosphate plowed down in the spring that a singificant response in yield was obtained from planting time fertilizers. Highest yields were obtained where 400 and 800 pounds had been applied as a plow-down application. These data show very clearly that the phosphorus fertility of the soil determines, for a large part, the yield response obtained by fertilizer application at planting time.

There was no over-all effect of fertilizer on percent sucrose. Beets from plots receiving 0 and 150 pounds of planting time fertilizer and no phosphate plowed down had the lowest sugar contents. There was no further evidence that an increase in sucrose content would result from additional fertilizer regardless of amount or method of application.

While the differences for percent purity were not statistically significant, there does appear to be a slight reduction in purity as the amount of planting time fertilizer was increased in

Table 3.—The effect of time and method of application of fertilizer on percent sucrose, purity and yield of roots, and gross sugar. (Monitor Sugar Co., 1959).

Lbs./acre P_2O_5 plowed down —spring	Lbs./acre 5-20-10 planting time	Tons/acre	Sucrose	Purity	Cwt gross sugar/acre
			percent	percent	
0	0	13.0	16.1	91.7	39.0
0	150	15.0	16.3	91.1	44.5
0	300	16.0	16.8	90.1	48.1
200	0	16.7	16.7	91.2	50.9
200	150	16.8	16.7	88.9	49.3
200	300	16.8	16.4	88.3	48.4
400	0	18.7	17.3	89.2	57.4
400	150	19.1	17.0	88.5	57.1
400	300	19.2	17.1	89.7	56.1
800	0	18.0	16.8	88.4	52.6
800	150	19.2	16.9	90.0	58.4
800	300	19.2	17.0	89.3	58.3
L.S.D. 5% level:					
P levels		1.92	NS	NS	
Planting time		0.60			

plots where 0 and 200 pounds of phosphate were plowed down. The gross sugar yields per acre were closely correlated with the tons of roots per acre.

Summary

The effect of planting time (5-20-10) fertilizer and rates of application of phosphate plowed under prior to planting on sugar beets was investigated on a Kawkawlin loam soil.

The data can be briefly summarized as follows:

1. There was a marked response of early growth, phosphorus content of tissue, and yield of beets to phosphate applications.
2. Planting time applications of 5-20-10 fertilizer increased early growth at each of the four levels of plow-down phosphate fertilization, increased the phosphorus and potassium contents of plants at blocking time, and increased the phosphorus content in the petioles of leaves where 0 and 200 pounds of phosphate were plowed under.
3. The percent extractable phosphorus in the green tissue decreased as the season progressed where no phosphate had been plowed down. The reverse of this situation occurred where phosphate had been plowed under.
4. There was no significant effect on the percent of sucrose or percent apparent purity due to the fertilizer application.

However, there appeared to be a decrease in apparent purity as the amount of planting time fertilizer was increased in the plots with 0 and 200 pounds of phosphate (P_2O_5) plowed down.

5. The treatments had no definite effect on the percentage of calcium or magnesium in the tissue, but at the last date of sampling where the highest rate of phosphate was applied, significant decreases in calcium content of the tissue were noted.

6. The effect of planting time 5-20-10 fertilizer in the growth of sugar beets was more marked at the lower rates of phosphate fertilizer plowed down. There did not appear to be any practical advantage of applying more than 150 pounds of fertilizer at planting time, providing there was a rather high level of phosphorus in the soil.

Hailstorm Damage to Crops in Northeastern Colorado and an Analysis of Precipitation Anomalies Associated with a Cloud-Seeding Program in 1959

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Introduction

The High Plains area of eastern Colorado and Wyoming, and western Nebraska and Kansas, suffers from a dual problem of frequent shortages of precipitation and frequent hailstorms that inflict severe crop losses and property damage. Within this region, development of irrigation water supplies from surface and underground sources has permitted the development of irrigated agriculture. A large part of the region, however, does not have a suitable source of water for irrigation, and must depend on precipitation for crop production.

The severity of the hail problem is indicated by Figure 1, which shows that this region suffers from the highest average annual number of days with hail in the United States (9)[2].

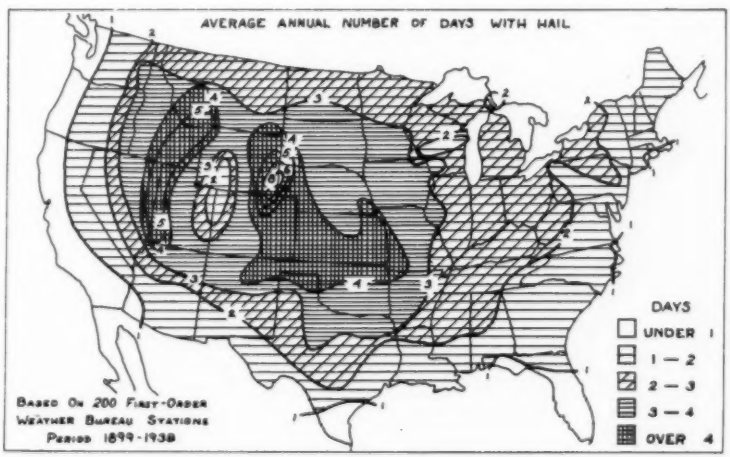

Figure 1.—Average annual number of days with hail for years 1899 to 1938. (Source: Climate and Man. Yearbook of Agriculture, U. S. Department of Agriculture, 1941, p. 730.)

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[1] Assistant Research Engineer, Engineer Research, Colorado State University, Fort Collins, Colorado.

[2] Numbers in parentheses refer to literature cited.

The economic cost of hail damage is reflected in current hail insurance rates. The cost for crop-hail insurance under a standard "10 percent deductible" insurance policy in this region ranges upward to $22.00 per $100.00 insurance for a crop such as wheat (3). (The rate for sugar beets is one-half that for wheat, reflecting a greater resistance to hail damage by the beet plants.)

A cloud-seeding program was used in 1959 in northeastern Colorado in an attempt to reduce hail damage. In a "target" area of about 3400 square miles clouds were seeded with silver iodide generators by a commercial weather modification company, using five aircraft and about 125 ground generators.

A study was made to evaluate this operation. This paper, however, is limited to background information pertaining to methodology of evaluation of changes in hail damage, and to presentation of data on precipitation anomalies associated with the cloud-seeding program.

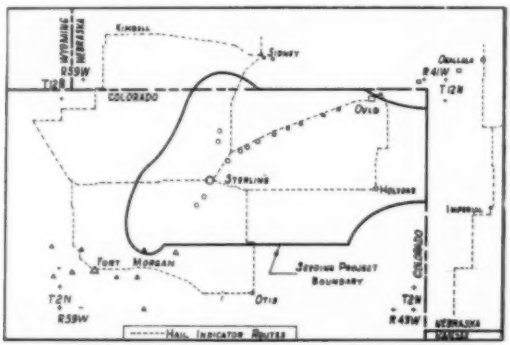

Figure 2.—Location of study area including The Great Western Sugar factory districts and associated beet dump areas. Δ is for the Fort Morgan factory district and associated beet dumps, O for Sterling, and □ for Ovid.

Procedure

Figure 2 shows the location of the target area and The Great Western Sugar Company Districts of Fort Morgan, Sterling and Ovid, Colorado. The data for the study were secured from observations in and bordering the target area.

During the study the following data were collected:

1. Information was obtained from a survey mailed to residents of the area living in or near Section 8 and 18 in each Township in Colorado between Township 3 to 12N and 42 to 59W inclusive. Cooperators were requested to report hail occurrences by mail, giving location, time of

hail occurrence, size distribution of hail, and concurrent weather phenomena including wind, lightning and precipitation amounts. A total of 389 reports of hail occurrences of this type were received between 15 May and 15 September 1959.

2. Data were obtained from periodic examination of approximately 250 hail indicating devices located in or near the target area. Figure 2 shows the routes along which the devices were located. A total of 358 indicators that had been damaged by hail were examined between 15 May and 15 September. Figure 3 is a schematic drawing of the hail indicating device.

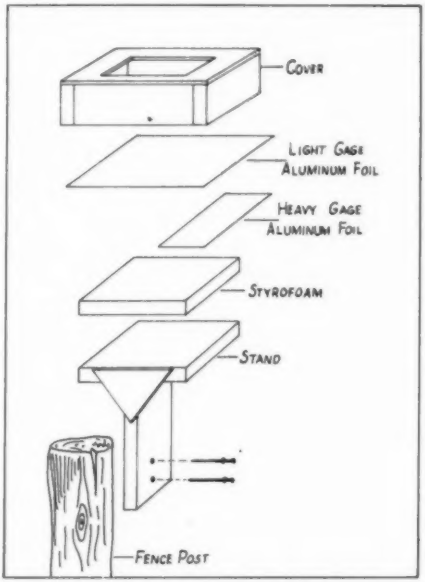

Figure 3.—Schematic diagram of hail indicating device.

3. Reports were obtained from the Weather Modification Company on locations and times of ground generator operation, and routes and times of seeding by aircraft.
4. Information on the amount and type of hail damage to sugar beets between 1929 and 1959 was obtained from the Ovid, Sterling, and Fort Morgan districts of The Great Western Sugar Company.
5. Reports of precipitation and other weather data were obtained from U. S. Weather Bureau cooperative observers in and near the area.

Results

A. Historical Records of Beet Damage

From the information in item 4 above the cumulative relative frequency of occurrence of light, medium, heavy, and total beet damage[3] was determined for The Great Western Sugar Company Districts of Ovid, Sterling and Fort Morgan[4]. The results are shown in Figure 4. Although the subjectivity of these measurements is recognized they are considered to be the best historical records of hail damage available.

Figure 4 shows that highest losses in 1959 were experienced in the Sterling factory district, with lowest losses in the Ovid factory district.

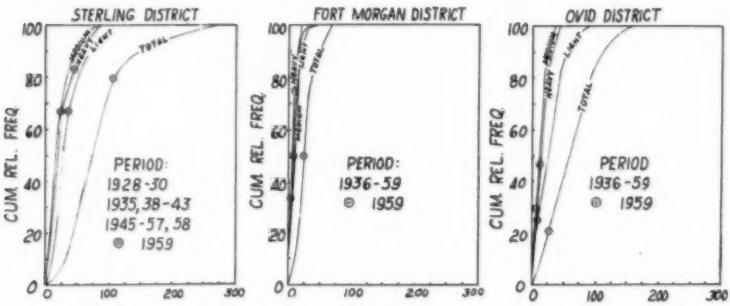

Figure 4.—Cumulative relative frequency in percent of hail damage to sugar beets in the Fort Morgan, Sterling, and Ovid, Colorado, The Great Western Sugar Company factory districts. Abscissa is the percent of acres planted that were damaged by hail during one season.

B. Hail-Precipitation Relations

Studies of hail in the Denver area by Beckwith (1) indicate that a relationship probably exists in the Denver area between the number of hail days and precipitation for the period from April to October. A rank correlation test (4) was performed to determine if a relationship might exist between percent of normal precipitation and percent of total acres of beets damaged.

The percent of normal precipitation for May-August for Sterling and Fort Morgan combined was correlated with the percentages of light, medium, heavy, and total damage to beets (in terms of percent of total acres planted[5]) for the Ovid,

[3] Great Western Sugar Company officials defined "light" damage as 25 percent or less leaf defoliation; "medium" as 25-50 percent defoliation; and 50 percent or more as "heavy" damage. Concerning the application of these standards one manager commented "of course, these gradations are subject to the individual subjective interpretations of each fieldman reporting the damage—and these variations are often as many as men who view the damage."

[4] The "Fort Morgan" district incorporates the former "Brush" district. Since the analysis was done on a percentage basis, a change of area does not affect the analysis.

[5] It should be noted that use of this definition permits hail damage in excess of 100 percent, since it is possible to experience damage at a point more than once during the season.

Sterling, and Fort Morgan factory districts of The Great Western Sugar Company. The results of this statistical test indicated that for all degrees of hail damage there was no significant relationship between percent of normal precipitation and percentage of beet acreage damaged. The results of this test are included in Table 1.

Table 1.—Results of rank correlation test between percentage of normal precipitation and percentage of beet acreage damaged by hail.

	N (Years)	r	$r_{.95}$
P vs. L	23	—0.035	0.352
P vs. M	23	0.278	0.352
P vs. H	23	—0.010	0.352
P vs. T	23	—0.059	0.352

where

P = percentage of normal precipitation (May to August) for Sterling and Fort Morgan combined.

L, M, H, T = percentages of beet acreages planted with light, medium, heavy, and total hail damage for Ovid, Sterling, and Fort Morgan factory districts of The Great Western Sugar Company.

r = rank correlation coefficient.

$$= 1 - \frac{6\,[\Sigma\,(Y_i - X_i)^2]}{N\,(N^2 - 1)}$$

Figure 5 is a scatter diagram of hail damage to beets in percent of acres planted and precipitation in percent of normal for the areas described. The line does not indicate any correlation but is merely drawn such that one-half the data are above the line. The years that fell above the line were then examined to find if they had a preponderance of hail days resulting from frontal or non-frontal meteorological conditions (5). From this analysis it was concluded that a preponderance of frontal or non-frontal hail days is not an important factor in determining the extent of hail damage. It may be noted that nine of the past thirteen years are positioned above the line on Figure 5. This indicates a possible inconsistency in the data. A possible explanation is that the system used to report beet damage may have been improved in the past few years.

C. Hail Intensity Measurements by an Energy Parameter

Early in the study, it was decided that a better statistic than number of days with hail was needed to determine any effects that cloud seeding might have on hail intensity and occurrence. Using indicators as shown in Figure 3, it was found that a satis-

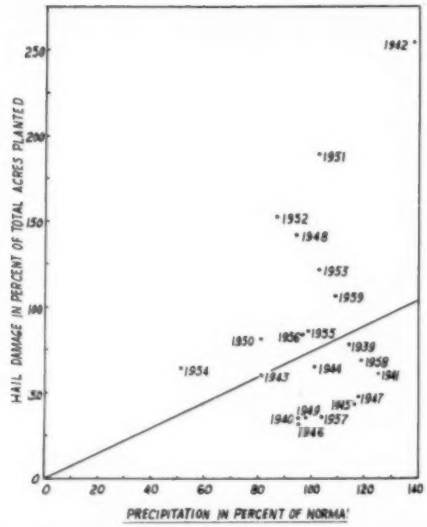

Figure 5.—Scatter diagram of total hail damage to sugar beets (Fort Morgan, Sterling and Ovid factory districts) vs. percent of normal precipitation (May-August) at Fort Morgan and Sterling.

factory approximation of the energy per square foot delivered by a hailstorm could be determined from the number and size of the dents left on the aluminum foils (6). This estimate of the impact energy from the hailstorm, in units of foot-pounds per square foot, is hereinafter called an "energy number."

During field use of these indicators, it was observed that medium crop damage was usually present when the energy number was between 50 and 100 ft-lbs per square foot. This is only approximate because crop damage is dependent not only on the intensity of the hail, but also on the type and stage of growth of the crop. For example, sugar beets are quite sensitive to hail damage during the seedling stage and become more resistant to hail damage as they mature. Wheat has different characteristics, being extremely sensitive to hail damage at maturity.

From reports of hailstone size, number, and attendant wind furnished by the cooperative observers, an approximation was made of the energy number of the hail that fell at the point of observation[6]. A rank correlation test (4) was made between this energy number[6] and the amount of precipitation concurrent

[6] This estimate was based on the laboratory calibration of the hail indicators, as described in reference (6).

with the hail. It should be noted that this test differs significantly from the one described previously. In the former test the variables, hail and precipitation, were compared by the use of average values covering large areas, while the second test compares these variables at a large number of discrete points. The results of this test indicate that there is a high probability of a positive relationship existing between the energy number of a hail occurrence and the precipitation delivered by the storm. This relationship appears to be present in both seeded and unseeded hailstorms. The results of the test are included in Table 2.

Table 2.—Results of rank correlation test between estimated energy per square foot from hail occurrences[1] and the attendant precipitation.

Month	Seeded cases		Non-seeded cases	
	N	r	N	r
May	85	0.183*	93	0.205*
June	54	—0.129	63	0.219*
July	47	0.383**	38	0.127*
Aug.	5	.90*	—	—

* = significant at the 95 percent level

** = significant at the 99 percent level

r = rank correlation coefficient

$$r = 1 - \frac{6\,[\Sigma\,(Y_i - X_i)^2]}{N\,(N^2 - 1)}$$

N = Numbers of pairs of observations

[1] Energy values were estimated from reports of number and sizes of stones and attendant wind reported by voluntary observers.

The energy number is a function of the number and size of stones and their fall velocities. If it is hypothesized that greater numbers of ice nuclei are available following cloud seeding, it should follow that the available water in the cloud would be distributed in a larger number of drops and that the diameter of hailstones would be reduced. If one hypothesizes that cloud seeding could thus reduce the diameters of the stones from d to d' without changing the total quantity of precipitation that falls as hail, then the energy number of a hail occurrence would be reduced. Figure 6 shows the effect of various assumed changes in hailstone diameter on the hail impact energy change ratio,

$\frac{E'}{EK}$, where

E′ = energy number resulting from hail after the hypothesized change in diameter of the stones from d to d′.

E = the energy number resulting from hail before the hypothesized change in diameter of the stones from d to d′.

K = a factor associated with the cloud seeding. If cloud seeding produces no change in the total amount of water appearing as hail, K would be equal to unity. (The value of K is not known.)

Figure 6 shows, for example, that if seeding reduced the hail diameter 10 percent (d′/d = 0.9) and at the same time increased the total amount of hail 10 percent (K = 1.1), the energy number that would result from the hail following the hypothesized changes would be essentially unchanged. The calculations for producing Figure 6 are explained in Table 3.

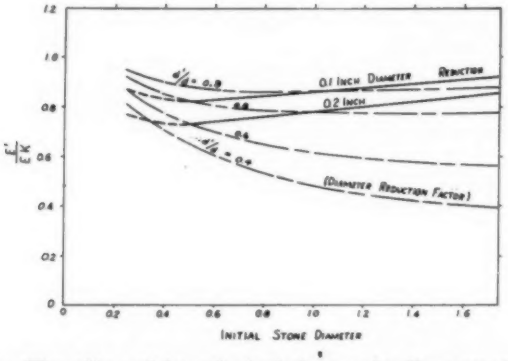

Figure 6.—The effect of hypothesized changes in diameter of hailstones on hail impact energy change ratio, (E′/EK). The 0.1 and 0.2 inch diameter reduction assumes that each stone is reduced by 0.1 or 0.2 inch from the initial diameter. The diameter reduction factors are the ratios of the assumed final diameter d′ to the initial diameter d.

The following analysis considers the effects of various hypothesized changes in hail intensity on damage to crops having differing susceptibility to hail damage. The basic data in the analysis are the observed frequencies of occurrence of energy numbers in northeastern Colorado, during the summer of 1959 for hail events not affected by cloud seeding. The energy num-

bers were obtained from measurements of number and sizes of dents from hail indictors (Figure 3).

Consider the area under the curve of the observed frequency distribution of energy numbers (not shown). For such a curve, the ordinate is in units of events per season, and the abscissa in units of energy per event, which can be considered the same as damage per event if the assumption is made that a crop suffers damage if the given energy number is exceeded. Each observed energy number in excess of the value for the threshold of crop damage then represents a unit of hail damage for that hail event.

Table 3.—Computations for Figure 6.

A plot of hailstone-terminal velociety in still air was prepared using the fluid mechanics formulas[1].

$$d = \left(\frac{3\, R^2\, \mu^2\, C_d}{2\rho'\, g\rho} \right)^{1/3} \quad \text{and} \quad R = \frac{\rho V d}{\mu}$$

where

R = Reynolds number
d = Diameter of hailstone
μ = Viscosity of air
C_d = Coefficient of drag
ρ' = Density of hail
g = Gravitational constant
ρ = Density of air

The total velocity, W, of the stone when it hits the ground was assumed tto be the vector sum of the terminal velocity in still air, V, plus the concurrent surface wind velocity.

The kinetic energy, T, of a single stone, was then computed as

$$T = 1/2\ mW^2$$

where

m = mass of stone
W = Velocity of stone

For one inch depth of precipitation and a given stone size the number of stones per square foot was computed. The number of stones multiplied by the kinetic energy per stone yields the energy number for the assumed depth of precipitation and a particular stone diameter. The impact energy change ratio was computed as the ratio:

$$\frac{E'}{EK} = \frac{\text{impact energy for stones of diameter } d' \text{ from one in. depth of water}}{\text{impact energy for stones of diameter } d \text{ from one in. depth of water}}$$

Figure 6 was computed for the case of zero surface wind. Results would be comparable for non-zero surface winds.

[1] Foster, D. S., and Bates, F. C. 1956. A Hail Size Forecasting Technique. Bull. of the Am. Meteor, Soc. 37(4): 125-141.

The area under the curve to the right of a particular energy number then represents

$$\frac{\text{damage}}{\text{event}} \times \frac{\text{events}}{\text{season}} = \frac{\text{damage}}{\text{season}}$$

The ratio of the area under the curve to the right of a particular energy number to the total area under the curve is defined as "Relative Damage." The particular energy number for which crop damage is assumed to begin is defined as the "Critical Energy Number," (E_c).

Figure 7 shows the relation between Relative Damage and Critical Energy Number. The line labeled $\Delta E = 0$ is based on the observed frequency distribution of energy numbers for 1959 for hail events affected by cloud seeding, and shows that a change in E_c from zero to 10 ft-lbs per ft^2 would reduce the Relative damage by about 50 percent.

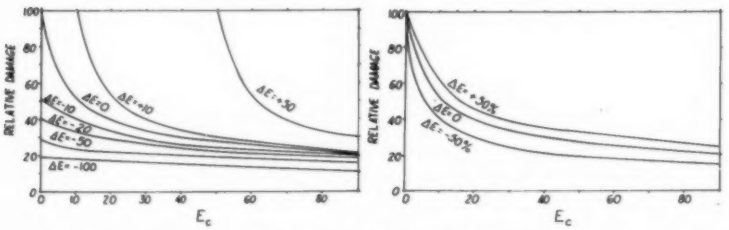

Figure 7.—Relative crop damage as a function of critical energy number (E_c) for various hypothesized changes in energy number associated with cloud seeding. Relative damage for the 1959 season (non-seeded cases) is given by $\Delta E = 0$. Hypothesized changes are (a) changing the energy number for each hail event by a fixed amount and (b) changing the energy number for each hail event by a fixed percentage.

Let us hypothesize that cloud seeding might change the observed frequency distribution of energy numbers. Two types of changes are hypothesized. For the first, a change in each energy number of a fixed amount is hypothesized. ($\Delta E = -10$, -20, -50, -100, $+10$, $+50$, ft lb/ft^2 in Figure 7a.) For the second, a percentage change in each energy number is hypothesized. ($\Delta E = \pm 50$ percent in Figure 7b.) The effects of such changes on the relations between Relative Damage and Critical Energy Number are shown in Figure 7. Figure 7a shows decided changes in relative damage for sensitive crops for a given hypothesized change in energy numbers, while for more hardy crops the change in Relative Damage is smaller for the same hypothesized changes in energy numbers. For hypothesized changes

of a fixed percentage, the effect on Relative Damage is greater at higher critical energy values (Figure 7b).

The foregoing analysis was based on the assumption that crop damage and energy number are directly related. While physically reasonable, there has not been an opportunity to study this problem in detail. Figure 8, developed from laboratory calibration of the hail indicators, shows the possible combinations of parameters that will produce a given energy number. The parameters are: the number of stones per square inch; the diameter of the stone in inches; and the surface wind velocity in miles per hour. From Figure 8 it may be seen that there is a wide variety of these parameters that can produce a particular energy number.

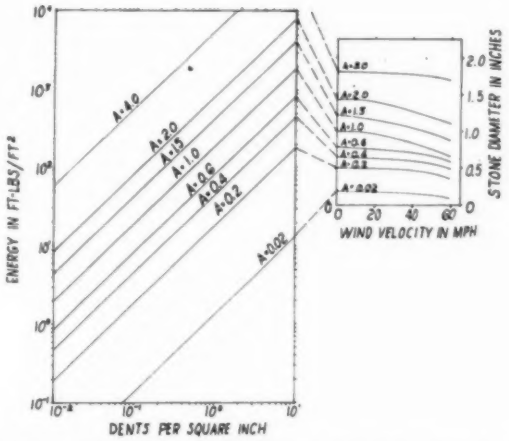

Figure 8.—Combinations of parameters that can produce a given energy number.

D. Precipitation Anomalies

The total precipitation in and near the target area for the months of May-August 1959, is shown in Figure 9. The percentage of normal precipitation for the same area for the same period is shown in Figure 10. Both figures were derived from data obtained from official U. S. Weather Bureau cooperative observers.

A "target-control" analysis was performed to attempt to detect differences in amounts of precipitation received in the target area as compared with adjacent control areas. The procedure used to obtain the transformed normalized precipitation variables τ_x (control) and τ_y (target) was identical with that

described by Thom (8), except that all storms in the historical record were used in the analysis. The transformed normalized values for the target were plotted vs. concurrent values for the control and fitted with a least-squares line. Confidence bands of ± 1, 2, and 3 standard errors were computed (7:p137).

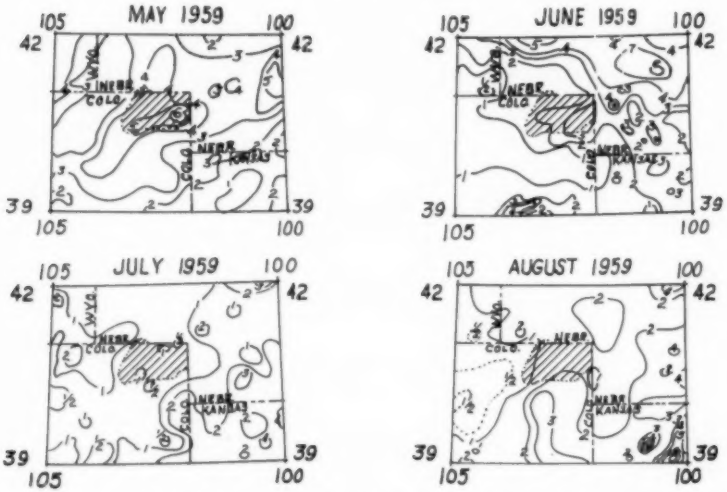

Figure 9.—Total observed precipitation in and near the study area during the months of May-August, 1959.

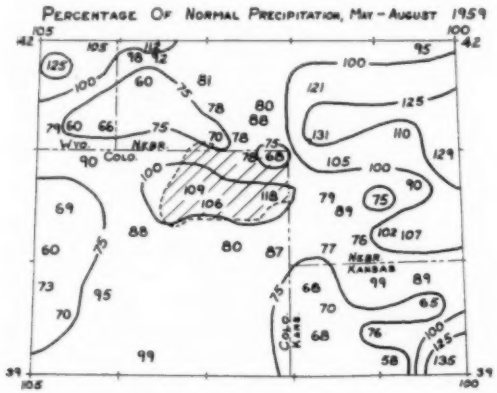

Figure 10.—Percentage of normal precipitation in and near the study area for the period May-August 1959.

Table 4.—Source of data used in target-control analysis.

Month	Target	Control	Years of record	Number of storms	Correlation coefficient
May	Sterling, Ovid, Leroy, Holyoke, Fleming	Greeley, Fort Collins, Fort Lupton	1942-48, 1950	26	0.77
June	Sterling, Ovid, Leroy, Holyoke, Fleming	Akron, Yuma, Wray	1942-44, 1944-50	40	0.96
July	Sterling, Ovid, Leroy, Holyoke, Fleming	Pine Bluffs, Kimball	1944-50	45	0.66
August	Sterling, Ovid, Leroy, Holyoke, Fleming	Akron, Yuma, Wray	1942-46, 1948-49	36	0.65

Eleven storms produced precipitation on the target and control stations between 15 May and 15 September 1959. The values of storm rainfall for these eleven storms were then superimposed on the diagrams. The results are shown in Figure 11, and summarized in Table 5.

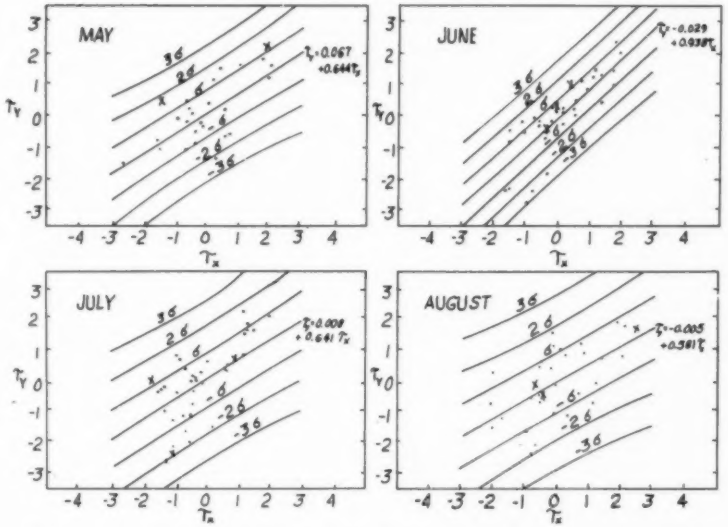

Figure 11.—Target-control analyses for precipitation anomalies. Values shown are the normalized transformed precipitation totals for control (r_x) and target (r_y) stations. Values for 1959 are marked "X."

Table 5.—Numbers of cases of storm precipitation in 1959 that fall within given standard errors of estimate from regression.

	-3σ	-2σ	-1σ	0	$+1\sigma$	$+2\sigma$	$+3\sigma$
May					2		
June				1	1		
July		1		1	1		
August			1	2			
Total[7]		1	1	4	4		

Although no single storm event departs by more than two standard errors from the regression, it is of interest to note that 8 out of 10 cases indicate a positive precipitation anomaly. In the absence of outside influences on the distribution of precipitation on the target and control stations, the chances of positive and negative anomalies would be equally likely. The probability of getting 8 or more positive anomalies out of ten cases by chance from an unbiased population is 0.0547.

Summary

A study was made of methodology of evaluating changes in hail damage and the effects of a cloud-seeding program on precipitation in northeastern Colorado, in 1959. Records of hail damage to beets from The Great Western Sugar Company factory districts were used as historical data to compare with the loss experience in 1959.

Statistical tests failed to reveal a significant degree of correlation between seasonal precipitation over the study area and hail damage to beets.

An energy parameter (units: ft lbs per ft^2) was used to describe hail impact energy resulting from a hailstorm. A significant positive correlation was found between this parameter and concurrent precipitation at a large number of discrete points.

If it is hypothesized that cloud seeding produces a larger number of smaller hailstones without changing the total quantity of precipitation which falls in the form of hail, then the hail

[7] One value for 1959 fell on the regression line.

impact energy from a given hailstorm will be decreased. This decrease, however, might be offset if seeding were to produce a greater quantity of precipitation falling in the form of hail.

If it is hypothesized that cloud seeding can reduce the hail impact energy of each hail event by a given amount, then the greatest benefits from such seeding would accrue to highly sensitive crops. If it is hypothesized that cloud seeding can produce a percentage reduction in hail impact energy of each hail event, the greatest benefits accrue to the more sturdy crops.

The assumption that hail damage is proportional to hail impact energy needs verification from further studies, since a given value of hail impact energy can result from a wide range of parameters including numbers and sizes of stones, and attendant wind.

Precipitation in the southern half of the target area for the period May-August 1959 was higher than normal and higher than adjacent areas, except to the northeast.

A target-control analysis for the season indicates that 8 out of 10 storms showed positive precipitation anomalies associated with the cloud-seeding operation. While results from individual storms are not significant, the probability of getting these observed numbers of positive anomalies for individual storms by chance from an unbiased population is 0.0547.

Acknowledgments

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The assistance of The Great Western Sugar Company officials and residents of the study area who supplied information and voluntary reports is gratefully acknowledged.

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Mr. T. J. Henderson assisted the study by supplying operational data from the Weather Modification Company.

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Studies on Backcross Generations and Advanced Generations of Interspecific Hybrids between B. vulgaris and B. webbiana

R. T. Johnson and G. W. Wheatley[1]

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In the quest to develop sugar beets with resistance to the sugar beet nematode, *Heterodera schachtii*, several investigators (1, 2, 3, 4, 5, 6, 7)[2] have attempted to make crosses between sugar beets and one or more of the nematode resistant species of the section *Patellares* of the genus *Beta*. Most attempts have resulted in failure of the F_1 seedlings to survive. The reason for this failure of the F_1 seedlings to survive is that they are generally incapable of developing a satisfactory root through which they can draw nutrients and moisture from the soil.

Stewart (7) reported survival of some offspring from a cross between sugar beets and *Beta procumbens*, but this material was lost in later generations due to a combination of inviability of the seedlings and both male and female sterility in the resulting plants. Coe (1), in trying to improve the viability of F_1 seedlings of a cross between sugar beets and *Beta procumbens*, developed a grafting method using small seedlings of both cion and root stock to provide in effect an artificial root on which the F_1 seedlings might survive. Survival rate of the F_1 seedlings was about seven percent. Johnson (3) reported a method of grafting small seedlings of the F_1 plants of the cross between sugar beets and *Beta webbiana* to a well established root stock of sugar beets. This method resulted in 70% survival.

Other approaches to combining the genotypes of one of these wild-type species of the genus, *Beta*, with sugar beets have been reported. Gaskill (2) made a bridge hybrid which consited of initially crossing Swiss chard with *Beta webbiana* and later crossing the F_1 plants, which would survive on their own roots, to sugar beets. Oldemeyer, et al. (5) reported a similar type of cross between *B. maritima* and *B. procumbens* and then crossing the resulting F_1 plants, which also survived on their own roots, to sugar beets. Savitsky (6) has crossed tetraploid *B. patellaris* $\times$ tetraploid sugar beets. Crosses of this type produce some F_1 seedlings which grow readily on their own roots.

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[2] Numbers in parentheses refer to literature cited.

Methods and Materials

This paper deals with several generations of crosses between *Beta vulgaris* and *Beta webbiana*. These include the F_1, four backcrosses (B_1, B_2, B_3, and B_4) and the advanced generations of B_2. The main purpose in these crosses was an attempt to transfer the nematode resistance in *B. webbiana* to sugar beets. Seed was produced on male sterile sugar beet plants by supplying pollen from *B. webbiana*. Grafting, as described by Johnson (3), was used to prevent death of the F_1 plants. The surviving F_1 plants were pollinated with pollen from sugar beets. Of the F_1 plants that survived, none produced any pollen. Female sterility was extremely high but a few viable B_1 seedlings were produced. These B_1 seedlings also required grafting for survival. The B_1 plants more closely resembled sugar beets than did the F_1, but they still possessed many of the characteristics of the F_1 plants. All were pollen sterile and female sterility was again very high. The B_1 plants that survived the grafting technique were again pollinated with sugar beet pollen and seed was produced from which five B_2 plants grew. These five plants looked very much like sugar beets. They grew on their own roots, flowered and set seed. One of these plants produced viable pollen, but the other four were male steriles. Table 1 gives the description of these five B_2 plants.

The occurrence of these five plants which grew on their own roots made possible a nematode evaluation which had not been possible through the generations of grafting. The five plants were put in greenhouse flats in cylinders of soil which contained a high population of sugar beet nematode in a manner described

Table 1.—Description of five B_2 plants from a cross between *B. vulgaris* and *B. webbiana*.

Plant number	Description
N51	Indistinguishable from sugar beet in appearance. Small root, produced no pollen, flowered normally and set viable seed. No satisfactory nematode evaluation.
N52	Indistinguishable from sugar beet. Small root produced viable pollen and fertile ovules. Roots contained only one female nematode larvae up to third stage when planted in nematode infested soil.
N53	Indistinguishable from sugar beet, small root, no pollen, fertile ovules. Root contained 2nd and 3rd stage larvae and one dead 4th stage.
N55	Indistinguishable from sugar beet. Large root, no pollen, fertile ovules, susceptible to sugar beet nematode.
N56	Indistinguishable from sugar beet. Large root, no pollen, fertile ovules, suspectible to sugar beet nematode.

by Wheatley and McFarlane (8). As can be seen from Table 1, no satisfactory nematode evaluation was made on plant N51. Plants N52 and N53 appeared to show some resistance to the sugar beet nematode and plants N55 and N56 appeared to be completely susceptible.

Because one of the plants did produce pollen, it was possible to interpollinate the plants of this B_2 generation using plant No. N52 as the pollinator. It was also possible to self-pollinate plant No. N52 and to backcross one more generation to sugar beets, in this manner producing a B_3 generation.

Of the progenies produced from plants in this generation, ten had sufficient seed that some progeny testing could be made under greenhouse conditions for nematode resistance. Only crosses involving plants N51, N52 and N53, as one or both parents of the cross, were used. These plants were grown in nematode infested soil as described previously. Table 2 shows the results of nine of the progenies from these B_2 plants, plus a commercial check when grown in the greenhouse in soil infested with nematodes.

Under the conditions of this test, it can be seen from Table 2 that most of the progenies performed about the same as the open pollinated check variety, S-2, which is susceptible to the sugar beet nematode. There are some exceptions, however. The self-pollinated progeny of plant N52 appears to be excellent

Table 2.—Classification of sugar beet roots of ten progenies from a cross of *B. vulgaris* × *B. webbiana* when grown in greenhouse soil infested with sugar beet nematode, *H. schachtii*.

Types of cross:	Nematode class[1]				
	1	2	3	4	5
N51 × N52		3	7	1	1
N51 × A5601				2	1
N51 × SL340 (4N) Triploid		3	32	11	
N51 × A5218 (2N)			33	12	
N52 (selfed)	3	1	2		
A5218 (4N) × N52 Triploid		4	4		
A5218 (2N) × N52	1		8	12	1
N52 × A5218 (2N	2		9	10	2
N53 × N52				7	2
S-2 (Check)			7	4	1

[1] Classification from 1 to 5 indicate range from 1 (freedom from visible mature female nematodes on the beet roots) to 5 (large numbers of visible mature females on the roots.)

in that it produced no plants in classes 4 or 5 and half of the plants rated as No. 1. Other progenies also contained some very satisfactory plants.

From the ten progenies shown in Table 2, 14 individual plants were selected as being relatively free (class 1 or 2) from sugar beet nematode. As a further check on the visual evaluation of this material, root cuttings were sent to Dr. Morgan Golden of the USDA Station at Salinas, California, for a more accurate determination by staining. The results of his staining counts on these 14 selected plants are shown in Table 3.

Table 3.—Results of examination of stained roots of progeny several generations removed from a cross of *B. vulgaris* × *B. webbiana*.

Plant number	Development of the sugar beet nematode
1	6 larvae (2nd and 3rd stage)[1]; 1 male molting into last stage.
2	6 larvae (3rd stage); 2 males in advanced 3rd stage.
3	4 larvae (3rd stage).
4	2 larvae (3rd stage).
5	6 larvae (3rd stage); 1 molting male.
6	3 larvae (3rd stage).
7	67 larvae in all stages to maturity; females, some developing males and 1 mature male.
8	No nemas found.
9	No nemas found.
10	9 larvae (2nd stage) and 1 molting male.
11	No nemas found.
12	6 larvae in various stage to maturity; 2 mature males.
13	No nemas found.
14	26 larvae (2nd to 4th stage).

[1] Stages from 1 to 5 represent different stages of development in the nematode within the root from very tiny, immature larvae to adults, respectively.

Of the fourteen plants, four (No. 8, 9, 11 and 13) had no larvae in the roots. One plant (No. 7) was apparently misclassified on the basis of visual observation because it contained many larvae in all stages of development. The other plants (12 and 14) also appeared to have some advanced larvae in their roots. The remaining seven beets appeared to have only immature larvae in their roots and were considered to be the group of most value. Roots with advanced stages of larvae development could not be considered resistant and roots with no larvae in the roots are probably escapes because even the roots of the resistant parent, *B. webbiana*, are entered by the larvae of this

nematode; however, in this case the larvae are incapable of completing their life cycle.

The seven plants containing only immature larvae were allowed to interpollinate or were crossed back again to sugar beets as desired. This provided a B_4 generation and another advanced generation of B_2 plants. From progenies thus produced 55 were selected in 1958 to plant in progeny rows in a field plot uniformly infested with sugar beet nematode.

Each plot consisted of a single row 20 feet long. They were planted in July and represented the first field testing of this material in soil heavily infested with sugar beet nematode. These beets were indistinguishable in appearance from sugar beets and had excellent vigor. The roots were dug and examined in November of 1958. From the standpoint of nematode resistance, the performance of these roots was disappointing. None of the progenies was without mature female sugar beet nematode on the roots which was similar to the open pollinated standard check with which they were compared. Not a single beet in the entire plot was free from the female nematode cysts.

As a final evaluation of this material, a test was planned to determine the yielding ability of this interspecific hybrid material. For this test, three strains resulting from the interspecific hybrids were compared with an open pollinated check, A5218. Descriptions of the strains follows:

A5920, a composite of all progenies of the B_4 and advanced generations of *B. vulgaris* $\times$ *B. webbiana*.

A5921, a composite of 2N lines whose ancestry included crosses involving plant N52 in Table 2.

A5922, a composite of 2N lines whose ancestry included plants N51 and N53 in Table 2.

A5218, a locally adapted, widely used, open pollinated, check variety.

This test was planted April 29 and harvested August 19. The test consisted of eight replications of each strain. Each plot consisted of two rows, 30 feet long, which were harvested for yield. Two samples of ten beets each from each plot were used for sugar determination. The soil in which this test was planted was known to be heavily infested with sugar beet nematode, so heavy that crop failure would result in normal sugar beet strains planted in such soil.

The yield of the check variety, A5218, in this test was only 6.25 tons per acre. One of the interspecific progenies produced a yield significantly different from it. The low yield of the lines in the test was observed to be almost entirely due to sugar beet nematode. Large numbers of nematodes were found on all roots in the plot at harvest time. There were no significant differences among the sugar content of any of these lines.

In the B_2 and B_3 generations, some plants in these lines appeared to show some nematode resistance. Yet in the B_4 and the advanced generations of B_2, no nematode resistance could be observed in either greenhouse or field tests in any of this material. The reason for this at present cannot be readily explained. Future studies will concentrate on further propagation of B_2 and B_3 plants in an attempt to determine more about the nature and inheritance of resistance to the sugar beet nematode. The greenhouse evaluation method used in the B_2 and B_3 generations should also be studied for reliability.

One accomplishment of this test was the demonstration that crosses of this nature can be made in the genus, *Beta*, and plants can be recovered by backcrossing which are indistinguishable from the recurrent parent, in this case sugar beet. The problem here was that the goal of nematode resistance was not achieved.

Summary

1. Interspecific hybrids between *B. vulgaris* and *B. webbiana* were made, propagated and studied through several generations in an attempt to combine the desirable characteristics of *B. vulgaris* with resistance to the sugar beet nematode, *Heterodera schachtii*. From the data presented it can be seen this goal was not achieved.

2. Plants which appeared to show some resistance to the nematode in the B_2 and B_3 generations failed to produce progenies resistant to it.

3. After four backcrosses to sugar beets, the plants produced were indistinguishable from sugar beets in all characteristics evaluated or observed.

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Carbohydrate Metabolism of Sugar Beets I. Respiratory Catabolism of Mono and Disaccharides[1]

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Introduction

The respiratory activity of sugar beets is of interest to processors because of losses of sucrose from post-harvest beet roots during storage. Earlier, it has been reported (1)[3] that losses may be related directly to the respiratory activity of sugar beets. Stout and co-workers reported that the respiratory rate of sugar beets can be affected by temperature, bruising, CO_2 and oxygen contents of environmental atmosphere, surface area per unit weight, genetic varieties, and inhibitors (4, 5, 6). The nature of pathways of carbohydrate catabolism operative in beet roots is not yet elucidated.

In the present work the radiorespirometric method (2, 3) has been employed to examine the utilization of several C^{14} labeled monosaccharides and disaccharides by beet roots.

Materials and Methods

Sugar beets of the variety US 22/3 were shipped from Nampa, Idaho, to the laboratory by air immediately after harvest. Test samples, selected on the basis of uniformity in shape, weight and maturity, were cleaned thoroughly prior to experimentation. Each of the test samples weighed approximately 500 grams.

The C^{14} labeled compounds used in the present study were obtained from the National Bureau of Standards through the kind cooperation of Dr. H. S. Isbell and several other commercial sources. Purity of each of the labeled compounds was established by means of paper chromatography and radioautography.

The radioactive substrate in the form of an aqueous solution was administered to an intact root by means of the "well" method. According to this method, a cylindrical well 9.5 mm in diameter, was drilled into the crown of the root to a length of 1/3 of the beet by means of a sterilized glass tubing. Upon withdrawing the glass tubing from the root along with the tissue core inside the tubing, a well with a defined area was hence

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[2] A portion of this work is taken from the thesis presented by R. D. Barbour for the degree of Master of Science at Oregon State College, 1958.

[3] Numbers in parentheses refer to literature cited.

established. The radioactive substrate was then introduced into the well in the form of an aqueous solution having a given volume and prescribed concentration with respect to both amount and radioactivity. Approximately ⅔ of the previously removed tissue column was then inserted to cover the opening of the well and molten paraffin wax was applied to seal the entire opening.

Inasmuch as the size of the well is defined and the diffusion of the substrate solution into the root tissue is likely to follow a constant rate, one should, therefore, expect reasonably reproducible findings from replicate experiments. The nature of the substrate diffusion processes was examined by a radioautographic study of root sections following the administration of C^{14} labeled sucrose. It was observed that the diffusion of sucrose followed a defined and homogeneous pattern. In addition, satisfactory reproducibility of recovery data was realized with respect to respiratory $C^{14}O_2$ from a great number of beet roots administered with an equal amount of C^{14} labeled sucrose by the "well" method.

The utilization of monosaccharides and disaccharides by beet roots in respiration was studied by the radiorespirometric method reported earlier for similar studies with fruits (2, 3). The method relies on the examination of interval recoveries of respiratory $C^{14}O_2$ from a biological system utilizing C^{14} labeled substrate. The substrates employed in these experiments as well as their amounts and radioactivities are given in Table 1. In a typical experiment, labeled substrate was introduced into the beet root by the "well" method. The beet was then transferred into a respiration chamber, 8 liters in capacity and equipped with inlet and outlet tubes. CO_2-free air was introduced into

Table 1.—C^{14} Specifically labeled substrates used in radiorespirometric experiments.

Substrate	Weight, mg	Specific activity μc/mM	Total radioactivity μc
Sucrose-U-C^{14}	25	1.25	0.10
Maltose-1-C^{14}	20	18.00	1.00
Glucose-U-C^{14}	50	1.80	0.50
Fructose-U-C^{14}	10	18.00	1.00
Mannose-1-C^{14}	10	18.00	1.00
Galactose-1-C^{14}	10	18.00	1.00
Ribose-1-C^{14}	8	18.10	1.00
Glucuronate-6-C^{14}	20	6.77	0.70
Gluconate-U-C^{14}	28	2.31	0.28

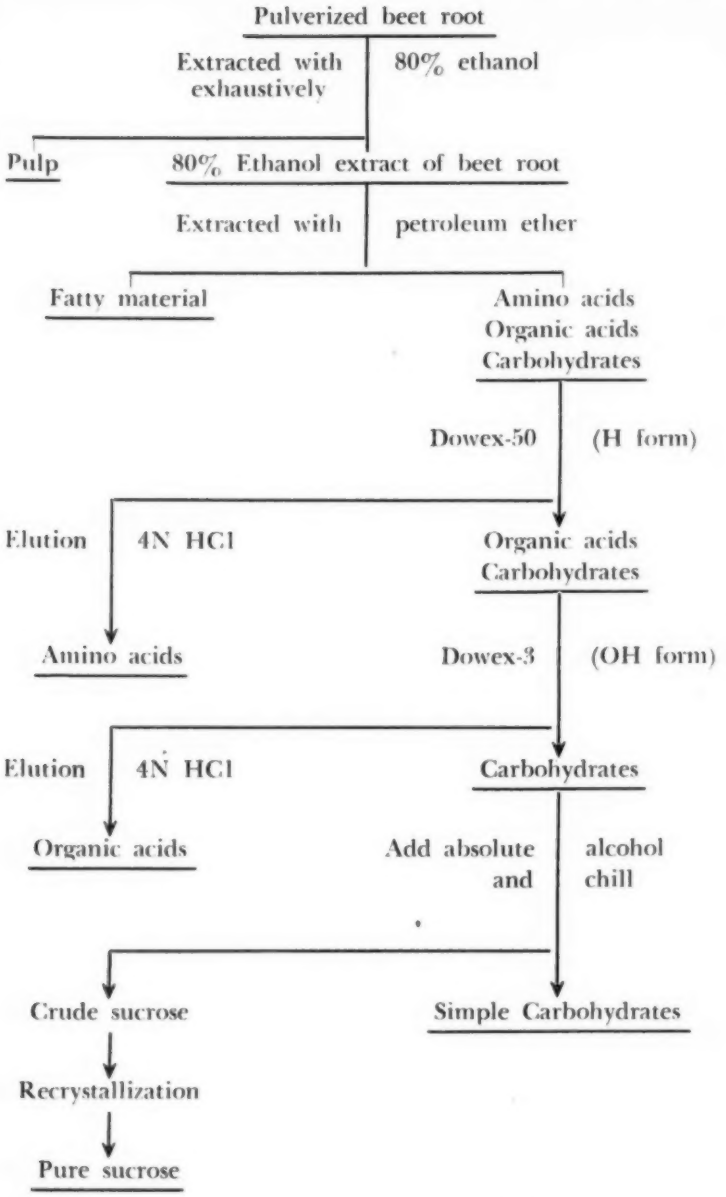

Figure 1.—Scheme for the isolation of beet constituents.

the chamber from a bypass manifold equipped with a flowmeter, thus permitting the control of air flow rate sweeping through the chamber. A flow rate of approximately 200 cc per minute was employed to insure a rapid turnover of atmosphere in the chamber. The respiratory CO_2 was swept into a CO_2 trap consisting of a sintered glass sparger immersed in 25 ml of 0.5 N CO_2-free sodium hydroxide solution. At prescribed time intervals, the solution in the trap was replaced and the CO_2 trapped was recovered as $BaCO_3$ by the addition of $BaCl_2$-NH_4Cl solution.

In a separate experiment, a relatively higher level of C^{14} labeled sucrose (9.1 μc in 100 mg) was administered to the beet weighing 831 grams, thus permitting the estimation of substrate incorporation into various constituents and metabolic products of beet root. Utilization of sucrose was followed radiorespirometrically for a period of 233 hours, at which time the specific activities of the respired CO_2 had declined and leveled to a steady value. The beet was then processed according to the scheme given in Figure 1 for the isolation of various groups of beet constituents for subsequent radioactive analysis.

The radioactivity of respiratory CO_2 and various beet constituents was determined by means of a Geiger-Muller gas flow counter. All counting samples were converted to $BaCO_3$, which in turn was mounted on aluminum planchets by the centrifugation technique. Countings were carried out to a standard deviation no greater than 1% and the counting data were corrected for background and self-absorption in the usual manner.

Results and Discussion

The radiorespirometric data on the utilization of C^{14} labeled monosaccharides and disaccharides given in Table 2 illustrates

Table 2.—Recoveries of $C^{14}O_2$ from intact beet root metabolizing C^{14} specifically labeled substrates.

Substrate	Cumulative percentage recovery of $C^{14}O_2$ at given hours				
	4 hr	8 hr	12 hr	16 hr	20 hr
Sucrose-U-C^{14}	0.3	0.4	8.6	11.5	13.6
Maltose-1-C^{14}	0.2	3.3	8.6	12.2	14.1
Glucose-U-C^{14}	1.5	5.8	9.2	12.4	14.7
Fructose-U-C^{14}	0.6	5.8	9.4	11.8	12.9
Mannose-1-C^{14}	0.1	0.6	1.5	3.0	4.2
Galactose-1-C^{14}	0.9	6.6	10.5	12.7	14.1
Ribose-1-C^{14}	0.5	4.6	11.4	18.5	21.8
Glucuronate-6-C^{14}	2.0	13.7	24.9	28.7	30.0
Gluconate-U-C^{14}	0.5	1.0	2.5	10.5	19.5

well the versatility of beet roots in metabolizing carbohydrate materials. At the end of 20 hours a considerable amount of substrate radioactivity was recovered in respiratory CO_2 from beet roots metabolizing C^{14} labeled sucrose, maltose, glucose, fructose, galactose, ribose, glucuronate and gluconate. Mannose was found to be metabolized only slightly indicating that mannose is not catabolized directly or converted rapidly to other readily catabolized hexoses such as glucose and fructose.

It is also noteworthy that 25% of the C^{14} activity in the administered glucuronate-6-C^{14} was converted to CO_2 in a matter of 12 hours. This fact may indicate the active operation of a direct decarboxylation process of glucuronate leading to the formation of xylose. Since a significant amount of $C^{14}O_2$ was produced in the gluconate-U-C^{14} experiment, it is possible that the phosphogluconate decarboxylation process may be operative in beet roots possibly as another mechanism for pentose production. It should be emphasized that the data presented here served only as evidence for the presence of enzyme systems responsible for the metabolism of the respective carbohydrates. Variation with respect to the rates of substrate diffusion in beet root does not permit one to elucidate the exact nature of the pathway nor to compare the findings with different labeled substrates.

The fate of sucrose in beet metabolism was traced by a radiorespirometric study of the utilization of sucrose-U-C^{14} with higher specific activity. The radiorespirometric data collected in this experiment are given in Figure 2. It is noted that the specific activity of $C^{14}O_2$ rises sharply in the earlier phase evidently reflecting the active metabolism of the substrate prior to its diffusion away from the site of administration. The progress of diffusion is indicated by the decline of the specific activity

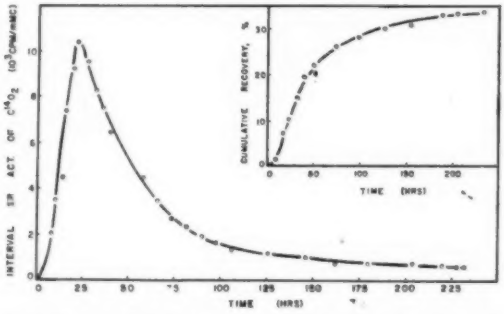

Figure 2.—Radiorespirometric pattern of sucrose-U-C^{14} catabolism in beet roots.

of $C^{14}O_2$ beginning at 25 hours after substrate administration and continuing until approximately 220 hours. The beet root was found to be still in normal physiological state and at the end of the experiment the final specific activity of the respiratory $C^{14}O_2$ was 619 cpm/mM of carbon. The beet root was then processed to isolate various fractions for radioactive assays. The distribution of substrate activity in these fractions are given in Table 3.

Table 3.—Distribution of activity of sucrose-U-C^{14} in beet root fractions.

Carbon dioxide	34.0%
Pulp	23.5%
Amino acids	5.6%
Organic acids	2.2%
Sucrose	11.2%
Neutral fraction (Simple carbohydrates, etc.)	13.2%

It is interesting to note that as much as 24% of the administered sucrose was found to be incorporated into the insoluble pulp fraction. The significance of this observation is yet to be elucidated. Examinatoin of the specific activity of beet sucrose in this experiment also permits one to correlate the origin of respiratory CO_2 to sucrose catabolism. The activity of sucrose was found to be 528 cpm per mM of carbon, a value that approaches the observed specific activity for the respiratory CO_2 (619 cpm/mM of carbon) at the end of the experiment. This fact led us to believe that sucrose is the principal, if not the exclusive, source of carbon for the production of respiratory CO_2 in beet roots.

The conclusion is drawn from the following considerations: (a) toward the end of the sucrose-U-C^{14} experiment, the specific activity of $C^{14}O_2$ has reached a steady level (Figure 2) and hence represents the specific activity of the carbon atoms of the carbonaceous compounds directly involved in the respiratory mechanism; (b) despite the fact that it is unlikely that the administered labeled sucrose had mixed homogeneously with beet sucrose, the specific activity of $C^{14}O_2$ reflected directly the $C^{14}O_2/C^{12}O_2$ ratio of the entire beet root under study; (c) the specific activity of sucrose isolated in the end of the experiment represented the sucrose-U-C^{14}/sucrose-C^{12} ratio of the entire beet root under study; (d) if the respiratory CO_2 is derived from carbon sources other than sucrose one would expect a noticeable dilution of $C^{14}O_2$ from $C^{12}O_2$ resulting in a significant re-

duction in the specific activity of $C^{14}O_2$ below that of the beet sucrose. The finding is in agreement with that reported earlier by Barr et al. (1) using a different method of estimation.

The nature of the pathways involved in the catabolism of beet sucrose has been studied and the findings are reported elsewhere (7).

Summary

In beet roots, sucrose is the most important carbon source for the production of respiratory CO_2. Simple carbohydrates and their derivatives such as maltose, glucose, fructose, mannose, galactose, ribose, glucuronate and gluconate can also be catabolized by sugar beet root.

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Carbohydrate Metabolism of Sugar Beets
II. Catabolic Pathways for Acetate, Glyoxylate, Pyruvate, Glucose and Gluconate[1]

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Introduction

In a previous paper (3), the utilization of sucrose, fructose, mannose, galactose, ribose, glucuronate and gluconate in the respiratory functions of sugar beets was demonstrated by a series of radiorespirometric experiments. However, the nature of the pathways for the catabolism of carbohydrates is yet to be elucidated. In the present work, C^{14} specifically labeled acetate, glyoxylate, pyruvate, glucose and gluconate were used as substrates to identify and estimate major catabolic pathways operative in sugar beets.

Materials and Methods

Sugar beet roots of the US 22/3 variety were used exclusively as test samples, and they were selected on the basis of uniformity in shape, weight, and maturity. Average weight of cleaned beet roots was approximately 500 grams.

The C^{14} specifically labeled acetate, pyruvate and glucose were obtained from the National Bureau of Standards through the kind cooperation of Dr. H. S. Isbell and from commercial sources except in the case of glucose-3 (4) -C^{14} which was prepared in this laboratory by the method of Wood et al. (16). Glyoxylate-1 and -2-C^{14} was prepared by the OsO_4 oxidation method of Weissbach and Sprinson (14) from fumarate-1,4-C^{14} and fumarate-2-3-C^{14} respectively. Potassium gluconate-1, -2, -3 (4), -6 and -U-C^{14} were prepared according to the method of Moore and Link (11).

The radiorespirometric experiments on the utilization of C^{14} specifically labeled substrates were carried out in a manner similar to that described previously (3). The "well" method (3) was employed exclusively as the means for the administration of labeled substrates.

Radioactivity of respiratory CO_2 samples were determined by means of a mica end-window Geiger-Muller counter using $BaCO_3$ exclusively as the counting form. Countings were carried out

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[2] A portion of this work is taken from the thesis presented by R. D. Barbour for the degree of Master of Science at Oregon State College, 1958.

to a standard deviation no greater than 1% and counting data were corrected for background and self-absorption in the conventional manner.

Results and Discussion

The application of the radiorespirometric method for the identification and estimation of catabolic pathways operative in a given biological system relies heavily on the reproducibility of $C^{14}O_2$ recovery data from C^{14} specifically labeled substrates within a given series of experiments. It follows that the method employed for the administration of labeled substrates constitutes an important factor in this regard. The degree of reproducibility of $C^{14}O_2$ recoveries from the beet roots administered with specifically labeled substrates such as glucose is indicated by the data presented in Table 1. It can be seen that the average deviation among replicate experiments is reasonably small and the data are, therefore, valid for pathway participation studies on either the qualitative or the quantitative basis.

Table 1—Reproducibility of $C^{14}O_2$ yields from beet roots metabolizing specifically labeled glucose samples.

Substrate	Substrate weight, mg	Radioactivity, μc	Percent recovery at 20 hours
Glucose-1-C^{14}	50	0.5	15.8
Glucose-1-C^{14}	50	0.5	17.5
Glucose-1-C^{14}	50	0.5	14.6
Glucose-2-C^{14}	50	0.5	13.0
Glucose-2-C^{14}	50	0.5	12.6
Glucose-2-C^{14}	50	0.5	13.0
Glucose-3,4-C^{14}	50	0.5	21.5
Glucose-3,4-C^{14}	50	0.5	21.0
Glucose-6-C^{14}	50	0.5	10.5
Glucose-6-C^{14}	50	0.5	12.2
Glucose-6-C^{14}	50	0.5	10.1

Identification of Catabolic Pathways.

In view of the extraordinary abundance of sucrose in beet root, and the role played by beet sucrose and in beet respiration (3), it is reasonable to believe that an active mechanism must be operative for the degradation of sucrose to CO_2 with glucose and fructose serving as the obvious intermediates. Efforts were, therefore, made to elucidate the catabolic pathways responsible for the conversion of glucose to smaller fragments, for biosynthetic functions, and to the respiratory CO_2 for energy production.

In studies of this type it is often advantageous to first understand the mechanism involved in the catabolism of degradation products derived from glucose such as pyruvate, acetate, and glyoxylate. The radiorespirometric pattern, i.e., time course plots of interval $C^{14}O_2$ recoveries or cumulative $C^{14}O_2$ recoveries for beets metabolizing C^{14} specifically labeled pyruvate are given in Figure 1. The extensive conversion of C-1 of pyruvate to CO_2 undoubtedly reflects the oxidative decarboxylation of pyruvate giving rise to the formation of a C_2 compound. That the C_2 compound is in the nature of acetate can be concluded from a comparison of the radiorespirometric patterns for C-2 and C-3 of pyruvate with that for C-1 and C-2 of acetate given in Figure 2. Not only do the time courses of the respective plots bear a close resemblance to each other, the ratio of recoveries for C-2/C-3 of pyruvate at the end of experiment was calculated as 3.0, a value approaching closely the analogous ratio of C-1/C-2 for acetate, 3.5. The exact nature of the mechanism for acetate catabolism cannot be defined by the present work, however, the preferential conversion of C-1 of acetate to CO_2 as compared to that of C-2

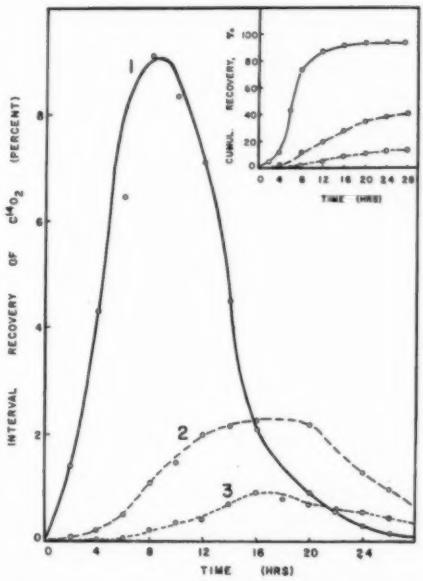

Figure 1.—Radiorespirometric patterns for sugar beets metabolizing C^{14} specifically labeled pyruvate, pyruvate-1-C^{14} _____, pyruvate-2-C^{14} ....., pyruvate-3-C^{14} .....

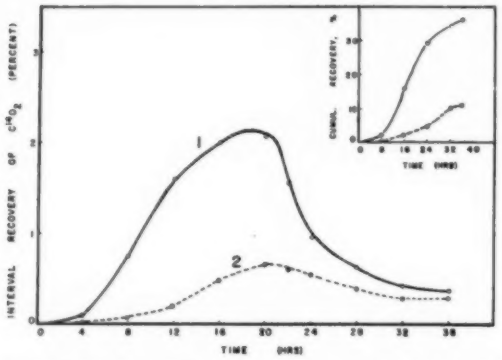

Figure 2.—Radiorespirometric patterns for sugar beets metabolizing C^{14} specifically labeled acetate. Acetate-1-C^{14}_____, acetate-2-C^{14} . . . . . .

suggests that the tricarboxylic acid (TCA) cycle may have been the principal pathway. The observed ratio, 3.5, for C-1/C-2 of acetate in CO_2 is somewhat higher than what has been observed with microorganisms (12). This finding can probably be accounted for by considering the effect of isotope dilution upon various labeled TCA cycle intermediates from unlabeled beet constituents (13). In the present case, the effect is presumably more pronounced in view of the amount of carbohydrate reserve present in beet roots.

The radiorespirometric patterns for the utilization of glyoxylate-1 and 2-C^{14} by beet roots are given in Figure 3. It is interesting to note that although preferential conversion of C-1 of

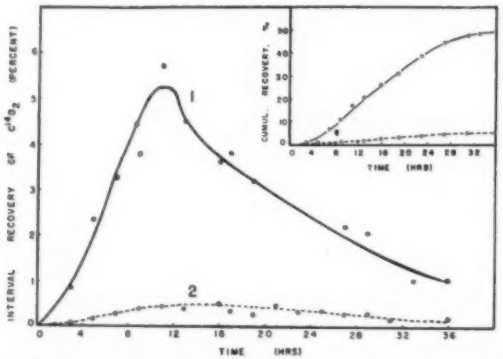

Figure 3.—Radiorespirometric patterns for sugar beets metabolizing C^{14} specifically labeled glyoxylate. Glyoxylate-1-C^{14}_____, Glyoxylate-2-C^{14} . . . . . .

glyoxylate to CO_2 is observed, the ratio of $C^{14}O_2$ recoveries C-1/C-2, for glyoxylate is found to be approximately 10, a value considerably higher than the analogous ratio observed in the acetate experiment. This fact may indicate that in addition to the operation of glyoxylate bypass pathway (9, 10), there may exist other pathways which are responsible for the preferential incorporation of C-2 of glyoxylate into beet constituents hence the preferential combustion of C-1 to CO_2. Possible mechanisms of this type, to cite a few, are the incorporation of C-2 of glyoxylate to a formyl group which is an important intermediate in C_1 metabolism.

With C^{14} specifically labeled glucose as tracing substrate, the radiorespirometric patterns for C-1, C-2, C-3,4 and C-6 of glucose are given in Figure 4. The prompt and extensive recovery of C-3,4 of glucose in respiratory CO_2 suggests strongly that the operation of the Embden-Meyerhof-Parnas (EMP) glycolytic pathway in conjunction with the oxidative decarboxylation of pyruvate is the major initial step for glucose breakdown. Meanwhile, the observed preferential conversion of C-1 of glucose to CO_2 in comparison with C-6 points to the participation of an

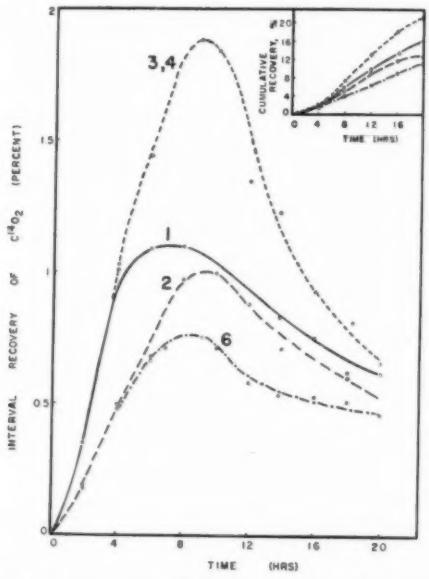

Figure 4.—Radiorespirometric patterns for sugar beets metaboliszing C^{14} specifically labeled glucose. Glucose-1-C^{14}_____, Glucose-2-C^{14} - - - - -, Glucose-3,4-C^{14} . . . . . , Glucose-6-C^{14} __·__·__.

alternate pathway, since these two carbon atoms should behave metabolically identical in the EMP-TCA scheme. The nature of the alternate pathway can be best analyzed with the discussion of the findings in the gluconate experiment.

It is reasonable to further speculate that the acetate, derived from glucose by way of the EMP-pyruvate decarboxylation mechanism, is catabolized via the TCA cyclic pathway as evidenced by the preferential conversion of C-2 of glucose to CO_2 relative to that of C-6. Conclusion is drawn from the fact that in the classical EMP-TCA scheme, one would expect to find that C-2 and C-6 of glucose corresponding to the carboxyl and methyl carbon atoms of acetate respectively. The possible operation of the TCA cycle in beet roots has been discussed earlier in connection with the acetate experiment.

The nature of the alternative pathway for glucose catabolism in sugar beets was further elucidated by the use of C^{14} specifically labeled gluconates as substrates in radiorespirometric experiments. Although the phosphogluconate decarboxylation pathway is known to occur in plant systems (1) the fate of the pentose phosphate, derived from glucose-6-phosphate, in plant catabolism has yet to be fully clarified. The use of C^{14} labeled gluconate offers the advantage that the catabolic behavior of pentosephosphates can be recognized without interference from the concurrent catabolism of substrate glucose since it is known that 6-phosphogluconate cannot be readily converted to glucose-6-phosphate directly (7).

The $C^{14}O_2$ recovery patterns for C-1, C-2, C3,4 and C-6 of gluconate are presented in Figure 5. The extensive conversion of C-1 of gluconate to CO_2, 90% after 36 hours, substantiates the occurrence of a C_1-C_5 cleavage pathway which is presumably in the nature of phosphogluconate decarboxylation. The catabolic fate of pentose derived from the latter pathway can be elucidated by examining the radiorespirometric patterns for the other carbon atoms of gluconate. The findings that $C^{14}O_2$ recoveries from gluconate carbon atoms are in the order of C-3,4> C-2>C-6 indicate that the pentose, presumably in the nature of ribose-5-phosphate, is converted rather extensively to fructose-6-phosphate through the action of transketolase and transaldolase. The conclusion is drawn from the consideration that two-thirds of the C-3,4 of gluconate should remain in C-3,4 position of the re-formed hexose phosphate (4) which can be in turn converted extensively to CO_2 when it is subjected to the glycolytic catabolism (5).

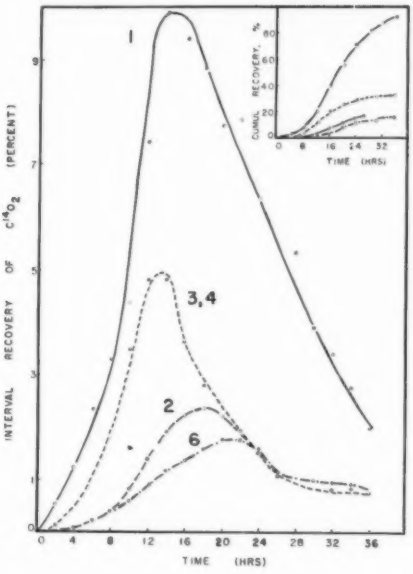

Figure 5.—Radiorespirometric patterns for sugar beets metabolizing C^{14} specifically labeled gluconate. Gluconate-1-C^{14}_____, Gluconate-2-C^{14} _____ _____, Gluconate-3,4-C^{14}....., Gluconate-6-C^{14}__.__.__.

The catabolism of glucose via the pentose cycle pathway has been recently examined by Katz and Wood (8, 15). These authors evaluated the effect of recycling of hexose monophosphates upon pathway participation under the assumption that the fructose-6-phosphate formed via the pentose cycle is in perfect equilibrium with glucose-6-phosphate and is hence engaged exclusively in repeated operations of the pentose cycle. The assumption thus implies that the re-formed fructose-6-phosphate would not be catabolized glycolytically by way of fructose-1, 6-diphosphate, a situation which cannot be accepted without experimental evidence particularly with biological systems wherein the EMP pathway is one of the major pathways.

A more realistic approach has been employed by Dawes and Holms (6) for the estimation of pathway participations. In a study with *Sarcina lutea*, these authors evaluated the relative contribution of the EMP pathway and the pentose cyclic pathway on the basis that thee fructose-6-phosphate formed in the latter pathway is in equilibrium with both glucose-6-phosphate and fructose-1,6-diphosphate and hence should display catabolic be-

haviors identical to that of substrate glucose. The treatment, although derived only from theoretical considerations, appears to be plausible when one takes into consideration the respiratory and biosynthetical functions of glucose pathways. The radiorespirometric data presented in Figure 5 renders experimental proof to the assumption made by Dawes and Holms (6).

Estimation of Pathways.

If one may assume, on the basis of the foregoing discussion, that in beet root glucose is catabolized primarily by way of two major pathways in the manner given in Figure 6 (4, 6), it would then be possible to make use of the cumulative $C^{14}O_2$ recovery data from the glucose experiments to estimate the relative participation for each of these two pathways. The principle of the method involved has been presented by Barbour et al. (2) for fruit studies.

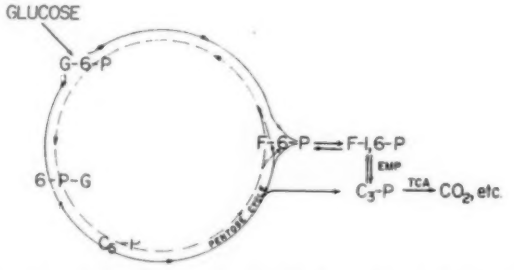

Figure 6.—Fate of glucose in beet catabolism. G-6-p glucose-6-phosphate; F-6-p, fructose-6-phosphate; 6-PG, 6-phospho-gluconate; C^5-P, pentose phosphate; C_3-P, triose phosphate; EMP, the Embden-Meyerhof-Parnas pathway; PC, the pentose cycle pathway; TCA, the tricarboxylic acid cycle pathway. Main pathways _____, Pentose cycle recycling ..... .

The necessary assumptions required for the estimation of pathway participation are essentially the same as that described previously for fruit studies (2). In short, these assumptions can be summarized as follows: (a) The administered glucose is catabolized extensively by way of the EMP glycolysis and the phosphogluconate decarboxylation pathway; (b) The trioses formed in the glycolytic pathway are equivalent to each other at the pyruvate level with respect to further metabolic reactions; (c) The pyruvate derived from the trioses is decarboxylated promptly and extensively giving rise to acetate which is in turn catabolized by way of the TCA processes; (d) The conversion of C-1 of glucose to CO_2 in the phosphogluconate decarboxylation is a prompt and practically quantitative process; (e) The pentose

formed in the phosphogluconate decarboxylation does not contribute promptly to CO_2 production particularly in the earlier stage of the time course experiments. The evidence supporting these assumptions has been presented earlier in the discussion of the nature of catabolic pathways functioning in beet root.

In the estimation of pathway participation in plant systems, it is essential to first estimate the exact amount of the labeled glucose catabolized, since the administered labeled glucose was utilized only in part for catabolic functions. Bearing in mind the foregoing assumptions, one would expect that for each mole of glucose catabolized by way of glycolysis there would be formed one mole of respiratory CO_2 from either C-3 or C-4 of glucose and similarly one mole of CO_2 would be recovered in the respiratory CO_2 from C-1 of glucose catabolized via the phosphogluconate decarboxylation pathway. The latter is superimposed on the recovery of C-1 of glucose in CO_2 via the glycolytic pathway which is identical with that derived from C-6 of glucose. Consequently the percentage fraction of labeled glucose catabolized by beet root can be expressed as in Equation 1:

$$G_t = (G_1 - G_6) + G_{3(4)} \tag{1}$$

where G_t is equal to the percentage fraction of administered labeled glucose catabolized at time t and G_1, $G_{3(4)}$ and G_6 are the percentage cumulative radiochemical recoveries of the substrates in the respired CO_2 at time t from beet roots metabolizing equal amounts of glucose-1-C^{14} glucose-3, (4)-C^{14} and glucose-6-C^{14} respectively.

The estimation of pathway participation in the catabolism of glucose with respect to the fraction G_t of the administered amount is given in Equations 2 and 3:

$$G_p = \frac{G_1 - G_6}{(G_1 - G_6) + G_{3(4)}} \times 100 \tag{2}$$

$$G_e = 100 - G_p \tag{3}$$

where G_p represents the percentage fraction of administered glucose catabolized via the phosphogluconate decarboxylation and G_e represents the percentage fraction of glucose engaged in the EMP glycolytic pathway.

The values of G_t, G_p and G_e calculated according to these equations along with the radiorespirometric data (average results from three replicate experiments) employed for these calculations are given in Table 2 for experiments extended to a period of 20

Table 2.—Pathway distribution in beet roots.

Time	% Cumulative radiochemical recovery of substrate activity in CO_2				% Glucose-C^{14} catabolized	% Pathway distribution	
Hrs.	G-1-C^{14}	G-2-C^{14}	G-3,4-C^{14}	G-6-C^{14}	G_t (Eq. 1)	G_p (Eq. 2)	G_e (Eq. 3)
2	0.5		0.4	0.3	0.7	36	64
4	2.2	1.7	2.0	1.2	3.0	33	67
6	4.3		4.1	2.4	6.0	32	68
8	6.5	5.6	7.5	4.1	10	24	76
10	8.6		11.2	5.5	14	22	78
12	10.4	8.3	13.7	6.5	18	22	78
14	12.0		16.2	7.9	20	20	80
16	13.5	11.3	18.2	8.9	23	20	80
18	14.8		20.0	10.2	25	19	81
20	16.0	12.9	21.3	10.9	26	19	81

hours. It is noted that the value of G_t rises sharply in the earlier stage of the experiment and levels toward the end of the experiment; meanwhile, the values of G_p decline sharply at the beginning and soon reached a steady value at 10 hours after substrate administration. The trend of diminution in G_p values in fact reflects much to the validity of assumptions set forth for the derivation of equation 1 and 2, particularly the assumption on the fate of pentose (assumption e). It can be readily seen that the terms G_6 and $G_{3(4)}$ in these equations were meant to represent the percentage conversion of C-6 and C-3 (4) of glucose respectively in CO_2 via exclusively the EMP-TCA pathway. This is indeed true in the early phase of the radiorespirometric experiment (up to a duration of six hours in Figure 4). However, as catabolism of the administered glucose proceeded, the contribution of CO_2 production via the pentose cycle pathway (Figure 6) to the terms G_6 and $G_{3(4)}$ became more pronounced, as shown in Figure 5, thus resulting in the diminution of the calculated values for G_p. On the basis of the foregoing understanding, it would appear that the values G_p calculated from the data collected in the early phase of the experiment are closer to the actual participation of the individual pathways.

It, therefore, appears that in sugar beet roots over one-half of the glucose engaged in catabolism is routed through the EMP-TCA pathway with the remaining portion degraded via the pentose cycle mechanism. The latter pathway presumably serves as the major avenue for pentose production. The possible involvement of pentose cycle reactions in the catabolism of pentose derived from 6-phosphogluconic acid has been discussed earlier.

Summary

The nature and participation of pathways for the catabolism of acetate, glyoxylate, pyruvate, gluconate and glucose in sugar beet roots has been studied by the radiorespirometric method. It appears that glucose is utilized mainly by way of the EMP glycolytic pathway and to a minor extent via the phosphogluconate decarboxylation route. The bulk of the pentose derived from glucose via the latter pathway is believed to undergo pentose cycle reactions giving rise to the reformation of hexose which is in turn catabolized in a manner identical to that of beet glucose. The pyruvate derived from glucose by way of the EMP mechanism presumably undergoes extensive oxidative decarboxylation which is in turn metabolized via the TCA cyclic pathway and to some extent the glyoxylate bypass pathway.

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Notes Section

Marlex Plastic Containers for Growing Sugar Beet Seedlings. Aluminum foil is being used for containers in growing sugar beet seedlings in the greenhouse[1]. A container 2″ x 2″ which is 7″ deep holds about the same volume of soil as a standard 4″ clay pot, but occupies only one fourth as much space. These containers have proven excellent for vernalization of seedlings which are to be transplanted to clay pots or to greenhouse beds and for screening individual seedlings for nematode resistance.

Aluminum foil containers have the following disadvantages: (1) If not reused, the foil has to be collected for disposal; (2) if reused, the pots need reforming and can generally be used only one more time before corrosion occurs through the foil; and (3) the foil is not stiff enough for convenient filling of the containers with soil. Aluminum foil is, however, inexpensive.

To overcome the disadvantages of aluminum foil, a search was made for a plastic which could be creased in forming. Marlex polypropylene plastic formed a nearly indestructible hinge when creased. Precut sheets of Marlex, 7″ x 9¼″, 15 mils thick, were obtained for less than 5 cents each.

A forming device was designed, as shown in Figure 1. It is comprised of a length of angle iron 1⅞″ wide, beveled on the front edge. The plastic is placed on the angle iron with the edge flush with the back of the angle iron. The plastic is hinged by forcing a piece of extruded aluminum angle, bent to about 50°, over the plastic at the beveled edge of the angle iron. A handle was fitted to the aluminum angle for ease of handling. Each of the four corners of the container is hinged in this way. A paper clip holds the 2″ x 2″ x 7″ deep container in shape until it is filled with soil.

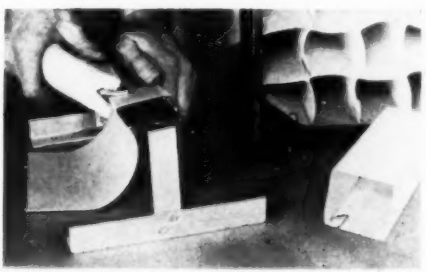

Figure 1.—Forming device for creasing Marlex plastic. Note ¾″ overlap held by paper clip on finished container.

[1] Mimeographed report to Beet Sugar Development Foundation by J. S. McFarlane, ARS, USDA, 1953.

Boxes for holding the containers are made by modifying a standard cantelope crate and are 22⅛" x 13⅛" x 7¼" deep inside dimensions. Sixty containers fit compactly into the box (see Figure 2).

Figure 2.—Illustration of the complete operation in forming containers and placement in boxes.

After use, the pieces of plastic are washed in our laboratory beet washer and are reused without further creasing.

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